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Antigen-Antibody Interactions

Teacher's Guidebook

(Cat. # BE-501)

MATERIALS INCLUDED WITH THE KIT

This kit has enough materials and reagents for 24 students (six groups of four students).

CHECKLIST

- 1 vial BE-Antigen 1
- 3 vials BE Antibody 1
- 1 bottle TAE Buffer (1X)
- 2 bottles Agarose
- 12 Glass Slides
- 6 Wide Bore Pipette Tips
- 30 Centrifuge Tubes (1.5ml)

SPECIAL HANDLING INSTRUCTIONS

- Store BE-Antigen 1 and BE Antibody 1 at 4°C.
- All other reagents can be stored at room temperature.
- Briefly centrifuge all small vials before opening to prevent waste of reagents.

The majority of reagents and components supplied in the *BioScience Excellence™* kits are non toxic and are safe to handle, however good laboratory procedures should be used at all times. This includes wearing lab coats, gloves and safety goggles.

For further details on reagents please review the Material Safety Data Sheets (MSDS).

ADDITIONAL EQUIPMENT REQUIRED

- Water Baths or Beakers and thermometer
- A small sandwich box
- Forceps

TIME REQUIRED

- Day 1: 1-2 hours
- Day 2: 0.5-1 hour

OBJECTIVES

- Understand specific properties of antigen & antibody.
- Antigen-antibody diffusion, interaction, and complex formation.
- Application of antigen-antibody interactions in research laboratories.

BACKGROUND

The key reaction of immunology and immune defense is the interaction of antibodies and antigens. This interaction is responsible for the body's defense against viral and bacterial infections and other toxins. The body's defense mechanism recognizes foreign substances, or antigens, and raises specific antibodies against them.

The antibodies bind to the antigens and form large macromolecular complexes. Large macromolecular complexes are formed due to the fact that each antibody can associate and bind with more than one antigen and each antigen can be bound by more than one antibody molecule. The formation of the large macromolecules results in their precipitation and the resulting precipitate is cleared by the body by various mechanisms. The interaction of antigen and antibody, resulting in precipitation, is also useful in research and diagnostics.

This study involves use of an immunodiffusion technique in which antigen and antibody are allowed to diffuse in solid agarose medium. Both the antigen and antibody diffuse freely through the agarose until they come into contact with each other and form a white precipitate. Antigen-antibody precipitate is formed in the zone where the concentration of the two matching pair reaches an optimal known as the zone of equivalence. Those regions of precipitation can be used for determination of concentration or titer of both antigen and antibody.

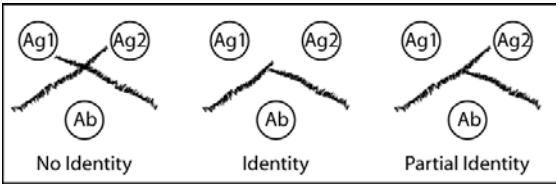
Two techniques use the principle of immunodiffusion.

1. The Radial Immunodiffusion (RID) technique

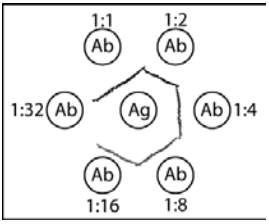
2. Ouchterlony Double Diffusion (ODD) technique.

Radial Immunodiffusion can quantitatively determine the concentration of an antibody. Basically, the antigen is incorporated into the molten agarose, which is then pipetted onto a glass slide and allowed to solidify. Once solidified small wells are punched into the agarose and known concentrations of antibody are loaded into the wells to act as standards. The unknown sample containing the antibody is also loaded. Next the antibody samples diffuse into the agarose in a circular, or radial, pattern. Due to antigen being in excess, diffusion occurs until a stable ring of antigen-antibody precipitate forms. The line of precipitation is the site where the greatest number of complexes are formed, at the zone of equivalence. The diameter of the endpoint precipitation ring corresponds to the amount of antibody in the sample. So, by comparing the standards to the unknown the amount of antibody in the sample can be determined.

The Ouchterlony Double Diffusion technique is based on the same principle of precipitation, but differs from the RID technique in the fact that both the antigen and antibody diffuse through the agarose. The precipitation is linear when the edges of the circular diffusion patterns meet and achieve their zone of equivalence. ODD technique can be used to test the similarity between antigens, for example in a study of evolution. Antigens from different species are loaded into two wells and the known antibody is loaded in a third well located between and slightly below the antigen wells to form a triangle. Upon diffusion, three possible patterns can occur (see figure below). If the antigens have no similarity then the precipitation zones will cross (No Identity); if antigens have identical antigenic determinants the precipitin lines between the antigen wells and the antiserum well stop at their point of intersection (Identity); if some antigenic determinants are shared one of the precipitin lines between the antigen wells and the antiserum well stops at the point of intersection, whereas the other continues past it, indicating that the antigen samples have some, but not all, antigenic determinants in common (Partial Identity).



A second use of the ODD technique is in antibody titration. The ODD technique for antibody titration is a simple technique that allows the visualization of the concentration (titer) of an antibody in a test solution. A known amount of antigen is loaded in a central well and varying titers of antibody are placed in wells circling the antigen well. The titer is visualized when one titer has a precipitation line and the next does not (see figure below). The example below shows the antibody titer to be about 1:16.



This Antigen-Antibody Interaction kit includes the Radial Immunodiffusion experiment and the Ouchterlony Double Diffusion experiment to determine antibody titer.

TEACHER'S PRE EXPERIMENT SET UP

Wear heat protective gloves when making the agarose solution.

TAE buffer is a common buffer consisting of Tris, Acetate and EDTA (ethylenediaminetetraacetic acid) and is a suitable dilution buffer for immunodiffusion.

1. Add 700µl TAE buffer to the BE-Antigen 1 vial. Soak the antigen for 5 minutes with periodically vortexing to dissolve the antigen completely.

2. Label 6 tubes with "Antigen" and aliquot in 10µl antigen solution. Supply each group with one vial of "Antigen".

3. Add 65µl TAE buffer to each vial containing the BE-Antibody 1. Soak the antibody for 5 minutes with periodically vortexing to dissolve the antibody completely. Combine the four vials.

4. Label 6 tubes with "Antibody Std 1" and aliquot 25µl antibody solution into each vial. Supply each group with one vial of "Antibody Std 1".

5. Add 25µl TAE buffer to the remaining stock Antibody solution (step 3). Label 6 tubes with "Unknown" and aliquot 10µl into each tube. Supply each group with one tube of "Unknown".

6. Label 6 tubes with "Dilution Buffer", aliquot 100µl TAE buffer to each tube. Supply each group with one tube of "Dilution Buffer".

7. Add 25ml TAE buffer to each Agarose bottle. Just before the class activity begins, heat the bottle in 100°C water bath or microwave oven to completely dissolve the agarose.

   
   If using a microwave, use short 10-second bursts for at least 30 seconds, swirling bottles after each 10-second interval. Do not boil. Check the bottles to assure agarose has dissolved. Use heat protective gloves.

8. Pipette 0.5ml Antigen (step 1) to one bottle of agarose and label this bottle as "Antigen Agarose". Mix the antigen with agarose by swirling the bottle. Keep *both* agarose bottles warm in a 50°C waterbath until required to prevent the agarose solidifying.

9. The slides need to be incubated overnight in a moist environment. Use a tray or container with a lip and place a layer of moist tissue paper in the bottom of the tray to prevent the agarose drying out. Once all the slides are placed in the tray cover with plastic film (Saran wrap or Clingfilm). You will need enough room for 2 slides per student group.

MATERIALS FOR EACH GROUP

Supply each group with the following components. Several components will be shared by the whole class and should be kept on a communal table.

- 1 vial (10µl) Antigen
- 1 vial (10µl) Unknown Sample
- 1 vial (25µl) Antibody Std 1
- 1 bottle Agarose Solution (Shared with whole class) ***(keep in +50°C waterbath)***
- 1 bottle Antigen-Agarose Solution (Shared with whole class) ***(keep in +50°C waterbath)***
- 1 vial Dilution Buffer
- 2 Glass Slides
- 1 Wide Bore Pipette Tips
- 5 Centrifuge Tubes (1.5ml)

PROCEDURE

Wear heat protective gloves when working with hot agarose solution.

Prepare Antibody Standards

1. As a group, prepare the antibody dilutions that will be used as standards in both experiments.

2. Label five tubes with Std 2, Std 3, Std 4, Std 5, Std 6 and add 12µl Dilution buffer to these tubes.

3. Remove 12µl from the Std 1 labeled tube, which contains the antibody solution and add to the tube labeled Std 2. Vortex or alternatively pipette up and down to mix.

4. Remove 12µl from the Std 2 labeled tube and add to the tube labeled Std 3. Mix as before.

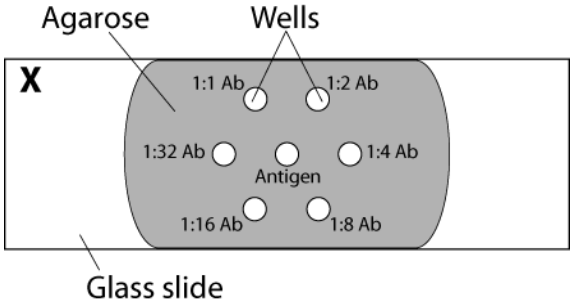
5. Repeat the serial dilutions by transferring 12µl from the Std 3 labeled tube and add to the tube labeled Std 4. Mix as before. Repeat by transferring 12µl from Std 4 to Std 5 and then Std 5 to Std 6 as before.

Ouchterlony Double Diffusion Antibody Titration

1. Each student group labels a glass slide with their group name and makes a small cross in the top left hand corner of the slide for easy orientation. Place slide on a flat surface.

2. Slowly, pipette 1.5ml Agarose Solution to the middle of slide. Spread the agarose to a large area with the pipette tip. Let the agarose polymerize for 10-20 minutes.

3. Place the slide on the template below and using the template as a guide carefully punch 7 holes (wells) into the agarose with the supplied Wide Bore Tip. **The wells should be no more that 10mm apart**. DO NOT DISCARD THE TIP.



4. Pipette 5µl Antibody Standards into the appropriate wells. Std1 is 1:1, Std 2 is 1:2, etc.

5. Carefully pipette 5µl Antigen into the center well.

6. Place the slides into a tray that has a layer of wet tissue paper.

7. Cover the tray and incubate overnight at room temperature.

8. The next day, hold the slides above a dark surface and observe the slides. Tilt the slide back and forth to aid the visualization of the bands.

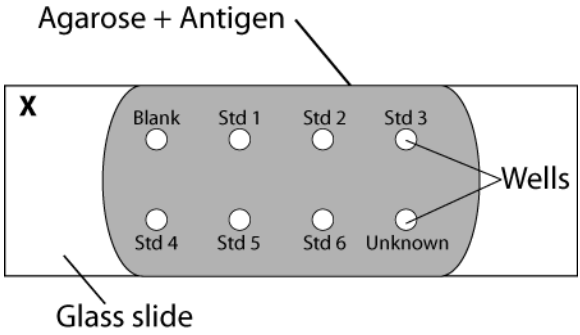
9. Draw a representation of your results in the Result section. The antibody titer is determined by visualizing the ratio of antibody before the titer where no precipitate is seen.

Radial Immunodiffusion Experiment

1. Each student group labels a glass slide with their group name and makes a small cross in the top left hand corner of the slide for easy orientation. Place slide on a flat surface.

2. Slowly, pipette ~2ml Antigen-Agarose Solution to the middle of slide. Spread the agarose to a large area with the pipette tip. Let the agarose polymerize for 20 minutes.

3. Place the slide on the template below and using as a guide carefully punch 8 holes (wells) into the agarose with the supplied Wide Bore Pipette Tip.



4. Pipette 5µl Dilution Buffer to the blank well, Antibody Standards and the unknown sample into their appropriate wells.

5. Place the slides into a tray that has a layer of wet tissue paper.

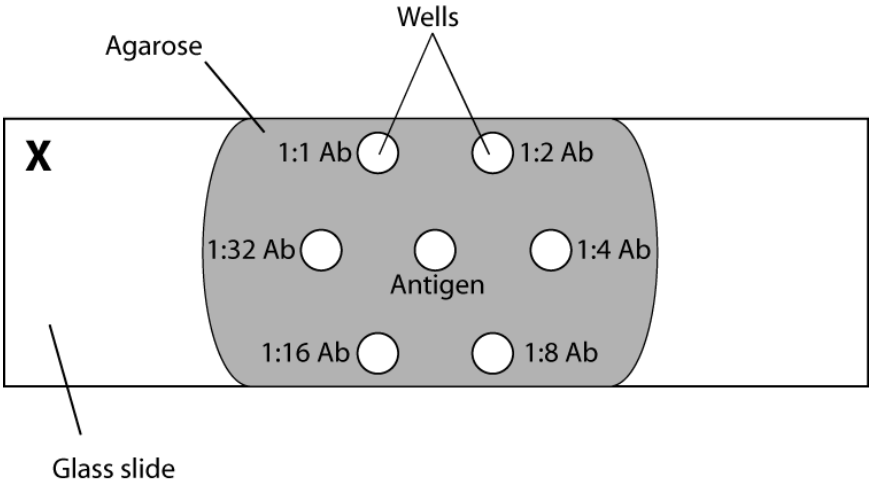
6. Cover the tray and incubate overnight at room temperature.

7. The next day, hold the slides above a dark surface and observe the slides. Tilt the slide back and forth to aid the visualization of the bands.

8. Measure the diameter of the white precipitate circle around the wells and plot a standard curve of circle diameter against antibody concentration and calculate the antibody concentration of the unknown sample. See results section for antibody concentrations.

RESULTS, ANALYSIS & ASSESSMENT

1. Draw a representation of the Ouchterlony Double Diffusion Experiment below:



2. Enter the diameter of the white precipitate circles below:

Antibody Standard Concentration (µg/µl)	Diameter (mm)
Blank	
Std 1 (0.4)	
Std 2 (0.2)	
Std 3 (0.1)	
Std 4 (0.05)	
Std 5 (0.025)	
Std 6 (0.0125)	
Unknown Sample	

3. Plot a standard curve of Diameter against Antibody Concentration. What is the concentration of the unknown sample?

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The antibodies bind to the antigens and form large macromolecular complexes. Large macromolecular complexes are formed due to the fact that each antibody can associate and bind with more than one antigen and each antigen can be bound by more than one antibody molecule. The formation of the large macromolecules results in their precipitation and the resulting precipitate is cleared by the body by various mechanisms. The interaction of antigen and antibody, resulting in precipitation, is also useful in research and diagnostics.

This study involves use of an immunodiffusion technique in which antigen and antibody are allowed to diffuse in solid agarose medium. Both the antigen and antibody diffuse freely through the agarose until they come into contact with each other and form a white precipitate. Antigen-antibody precipitate is formed in the zone where the concentration of the two matching pair reaches an optimal known as the zone of equivalence. Those regions of precipitation can be used for determination of concentration or titer of both antigen and antibody.

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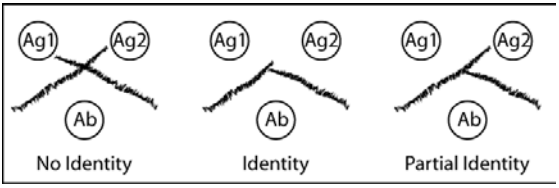

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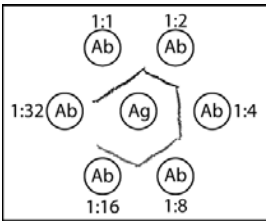

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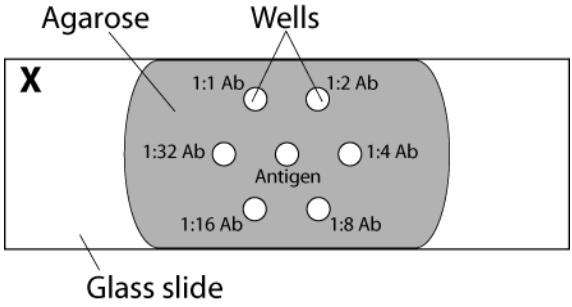

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7. Cover the tray and incubate overnight at room temperature.

8. The next day, hold the slides above a dark surface and observe the slides. Tilt the slide back and forth to aid the visualization of the bands.

9. Draw a representation of your results in the Result section. The antibody titer is determined by visualizing the ratio of antibody before the titer where no precipitate is seen.

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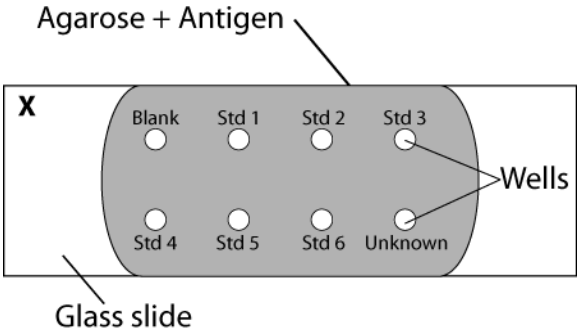

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8. Measure the diameter of the white precipitate circle around the wells and plot a standard curve of circle diameter against antibody concentration and calculate the antibody concentration of the unknown sample. See results section for antibody concentrations.

RESULTS, ANALYSIS & ASSESSMENT

1. Enter the diameter of the white precipitate circles below:

Antibody Standard Concentration (µg/µl)	**Diameter (mm)**
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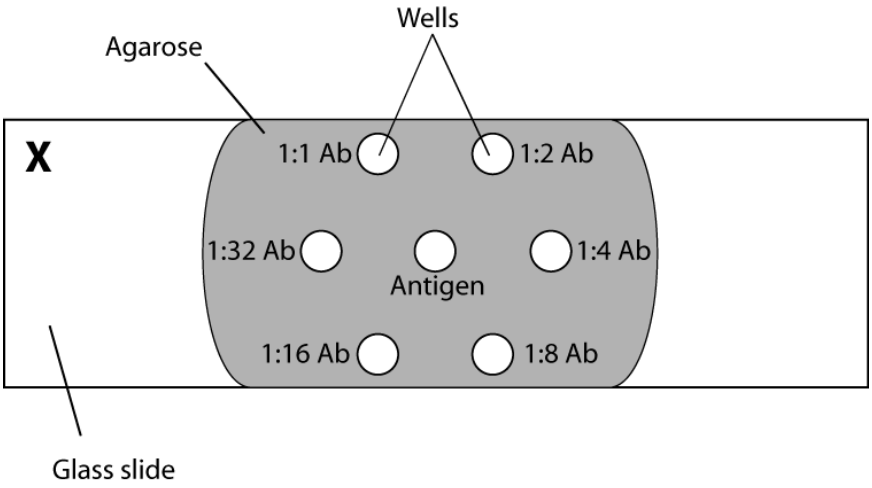
Std 1 (0.4)	
Std 2 (0.2)	
Std 3 (0.1)	
Std 4 (0.05)	
Std 5 (0.025)	
Std 6 (0.0125)	
Unknown Sample	

2. Plot a standard curve of Diameter against Antibody Concentration. What is the concentration of the unknown sample?

_____
_____
_____
_____

3. Draw a representation of the Ouchterlony Double Diffusion Experiment below:



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