ImLA Latex Agglutination

Learning Objectives

The student will

- Follow oral and written instructions and manage time in the lab efficiently.
- Apply correct terminology regarding microbiological techniques when making observations.
- Explain how immunological tests can be used to identify microbes and determine a person's blood type.
- Use agglutination to detect the presence of specific proteins in a sample.

Background/Theory

The **Latex Agglutination** test is a type of **indirect agglutination**. In indirect agglutination, the antigen is too small to be seen even in large aggregates. Visible clumping is artificially created by using large particles like latex beads.

When beads are coated with a specific antigen, agglutination is observed when the homologous antibody is present. See figure 1. This technique is most often used when probing for **IgM** antibodies, because their structure provides maximum cross-linking. One widely used example of this assay is a test for **rheumatoid factor (RF)** to confirm a diagnosis of rheumatoid arthritis. (OpenStax CNX, 2018)

Alternatively, to probe for a specific antigen, the beads are coated with the homologous IgG (antibody). The antigen may be displayed on the surface of a pathogen as shown in figure 2, or the antigen may be chemically removed from the cell and then exposed to the IgG covered latex beads.

Agglutination tests are widely used in underdeveloped countries that may lack appropriate facilities for culturing bacteria. For example, the Widal test, used for the diagnosis of typhoid fever, looks for agglutination of *Salmonella enterica* subspecies *typhi* in patient sera. The Widal test is rapid, inexpensive, and useful for monitoring the extent of an outbreak. (OpenStax CNX, 2018)

We will use latex agglutination to test for the presence of 6 of the Lancefield group specific antigens on the surfaces of microbes in the genus *Streptococcus*. In this particular test, you will not test the bacterial cells directly as shown in figure 2. Instead, you will first extract the antigens from the cell surfaces and then probe for the free antigen molecules in the solution. This allows us to test the sample with 6 different IgG coated latexes individually. The PathoDxtra kit can identify streptococci in Lancefield groups A, B, C, D, F, and G. The kit cannot identify streptococci that do not present any of the above listed Lancefield group antigens.

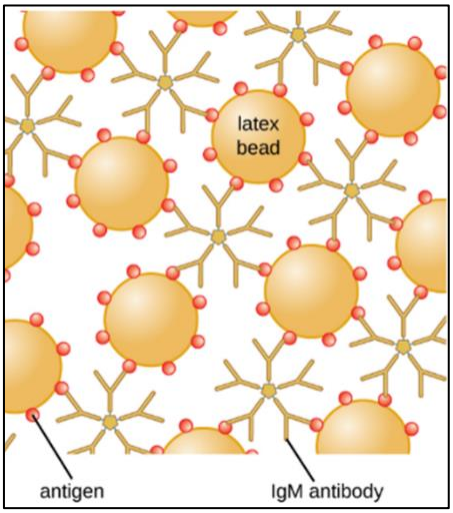

Figure 1 Latex beads coated with an antigen will agglutinate when mixed with patient serum if the serum contains IgM antibodies against the antigen. (OpenStax CNX, 2018)

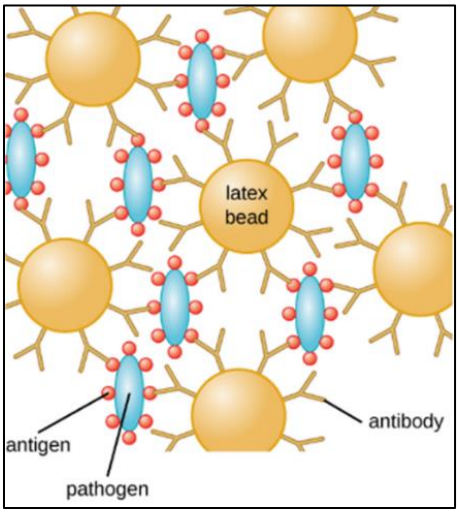

Figure 2 Latex beads coated with antibodies will agglutinate when mixed with patient serum if the serum contains antigens specific to the antibodies. (OpenStax CNX, 2018)

Lancefield Groups

In 1933, Rebecca Lancefield proposed a method for serotyping various β-hemolytic strains of *Streptococcus* species using an immunological assay called the precipitin test. She identified 5 groups, A through E based on specific cell-surface antigens (Lancefield, 1933). Following Dr. Lancefield's work, additional groups were identified using the designations F through V. Some species fall into 2 or more groups and some β-hemolytic strains do not have any of the identified antigens (Facklam, 2002). We will be concerned with Groups A, B, C, D, F and G because these groups contain the most clinically significant strains.

Streptococcus pyogenes belongs to the **Lancefield group A (Group A Strep or GAS).** This species is considered a pyogenic pathogen because of the associated pus production observed with infections it causes. *S. pyogenes* is the most common cause of bacterial pharyngitis (**strep throat**); it is also an important cause of various skin infections that can be relatively mild (e.g., **impetigo**) or life threatening (e.g., **necrotizing fasciitis**, also known as flesh eating disease). (OpenStax CNX, 2018)

S. agalactiae, **group B streptococcus (GBS)**, is an encapsulated gram-positive bacterium that is the most common cause of **neonatal meningitis**. *S. agalactiae* can also cause meningitis in people of all ages and can be found in the urogenital and gastrointestinal **microbiota** of about 10–30% of humans. (OpenStax CNX, 2018) They are also common inhabitants of oral and vaginal areas as part of the normal flora (Patterson, 1996).

Group C, *S. equisimilis* can cause endocarditis, bacteremia, pneumonia, meningitis and mild upper respiratory infections. (Patterson, 1996)

Enterococcus faecalis, a non-hemolytic streptococcus falls into **group D** as does *Streptococcus bovis*. Group D streptococci cause urinary tract infections, biliary (gall bladder related) infections, bacteremia, and endocarditis. (Patterson, 1996)

Streptococcus anginosus possesses both **group F and group G** antigens. It is the etiologic agent for some mild upper respiratory infections, endocarditis and subcutaneous or organ abscesses. (Patterson, 1996)

Other medically important Streptococci

Streptococcus pneumoniae, a normal member of the respiratory tract flora, is α-hemolytic and does not possess any of the Lancefield group antigens. As the name suggests, it is a cause of pneumonia. The best defined virulence factor is the polysaccharide capsule, which protects the bacterium against phagocytosis (Patterson, 1996). You may remember the classic experiments by Fredrick Griffith showing the non-encapsulated (non- lethal) form transforming into the capsulated, lethal form. (OpenStax Biology, 2018) The transforming factor was shown to be nucleic acid rather than a protein supporting the assertion that nucleic acids (RNA/DNA) are molecules that carry hereditary information.

Important members of the viridans streptococci, normal commensals, include *S. mutans* and *S. sanguis* (involved in dental caries), *S. mitis* (associated with bacteremia, meningitis, periodontal disease and pneumonia), and "*S. milleri*" (associated with pus forming infections in children and adults). (Patterson, 1996)

Experiment/Exercise

Materials per student pair

PathoDxra Kit containing latexes A, B, C, D, F, G, extraction reagents 1, 2 and 3
Reaction card
Sterile test tube
Pasture pipette and bulb

Sterile toothpicks

Cultures

Fresh overnight blood agar plate cultures of sample *Streptococci*, labeled W, X, Y or Z
Alternatively, you may sample a β-hemolytic colony from your throat sample streak plate

Procedure Lab 1

1. Watch the demonstration video. Use the results of the positive and the negative controls in the video as your results. Both solutions will be tested as if they are separate organisms and treated exactly like the cells you will test. The extraction reagents will be added to each control and then each control solution tested with each latex in the manner described below. The positive control is called **polyvalent** because many binding sites are present.
2. Prepare your materials
   a. Label each circle of the PathoDxtra reaction card with the letters A, B, C, D, F and G. (There may be extra circles on the card.)
   b. Make sure your PathoDxtra Kit has all the reagents.
   c. Make sure you have all the other materials as listed.
3. Extract the antigens from the cells. This step will allow you to test your sample with several different latex bead suspensions. (If you only need to test with one or two latexes, you could simply mix growth directly with the suspensions.)
   a. Add one full drop of extraction reagent 1 to an empty sterile test tube.
   b. With a sterile inoculating loop, aseptically remove 3-5 (more, if they are small) isolated β-hemolytic colonies from the plate. (This is one time when you need to be able to see growth on the end of the loop.)
   c. Suspend the cells in reagent 1 at the bottom of the test tube by gently agitating the loop in the liquid.
   d. Immediately, incinerate the loop upon removal from the test tube and before setting it down.
   e. Add one full drop of reagent 2 and gently mix the tube by flicking the bottom with your index finger for 5-10 seconds
   f. Add 5 drops of reagent 3 and mix as above for 5-10 seconds.
4. On one side of each circle on the reaction card, place one (1) drop of the corresponding antibody latex suspension. The latex beads tend to fall to the bottom of the container, so be sure to <u>shake each dropper well before dispensing</u>. Refrain from adding the cell extraction. See Figure 3.
5. With the cell extraction solution in the test tube, using the Pasture pipette, place one drop of the extraction solution next to <u>but not touching each</u> of the latex

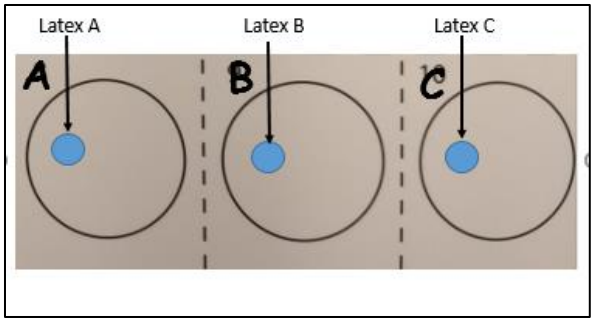

Figure 3. After shaking, place a drop of each latex suspension in the corresponding circle.

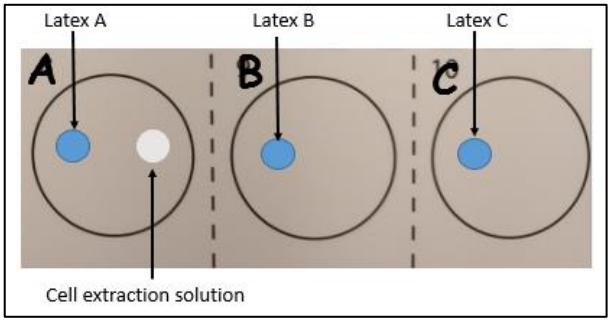

Figure 4. The cell extraction is placed in the circle so that it does <u>not touch</u> the latex suspension

drops on the card. See Figure 4. Leave the Pasture pipette in the test tube when finished.

6. Mix each latex with the cell suspension with a toothpick. Use a new toothpick with each circle/latex. Dispose of the toothpick in the bench top disposal container immediately after use. Do not set the toothpick on the bench top.
7. After all 6 latexes are mixed, gently rock the card back and forth. Look for agglutination within 30-60 seconds. It may take a bit longer than this. See Figure 5. When making observations keep in mind the following.
    - If you wait too long the solution may begin to dry on the reaction card leaving clumps of latex beads. You may erroneously assume that this is agglutination.
    - If you are unsure about your result, you may observe the card under the scanning objective of your microscope and compare a non-agglutinated result with the one in question.

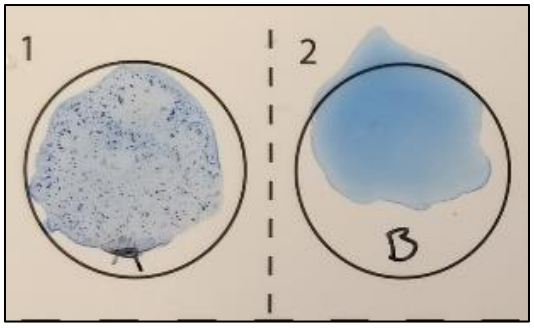

Figure 5. The granular appearance in the circle on the left is a positive agglutination reaction. The circle on the right shows no agglutination.

Lab Report: Latex Agglutination

Name _____
Lab Section _____

Data and Observations

Organism	Aggl w/ latex A +/-	Aggl w/ latex B +/-	Aggl w/ latex C +/-	Aggl w/ latex D +/-	Aggl w/ latex F +/-	Aggl w/ latex G +/-
Polyvalent Control						
Negative Control						

What conclusions can you make about your test organism? Can you identify it to the species? Why or why not? Can you make any conclusions about what it is not?

Post Lab Questions

1. Explain why the polyvalent control showed agglutination when mixed with each of the antibody latex suspensions?

2. If the polyvalent control had failed to show agglutination with the C latex, what specifically could it mean?

3. What makes the negative control solution, the negative control? (It may be more proper to ask, "What does it lack?") Why is it important to add the extraction reagents to it?

4. Figure 5 above shows agglutination with latex A. Draw what this interaction might look like on the molecular level. Include and label the latex beads, the IgG antibody, the antigen, the antigen binding site, the epitope. Make your drawing large and neat enough so that these regions can be seen clearly. (Your drawing will look similar, but not identical, to Figure 1 or 2 although you do not need to draw as many molecules.)

References

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