Lesson 2: Mix up with Osps

(Make sure you wash and dry the dropper bottles immediately after final use! The cornstarch gets moldy when it is left in the bottles. See page 6.)

Materials

To make blood samples with Lyme/OspA for the class
- 25 ml skim milk
- 75 ml water
- 16 ml cornstarch (~1 slightly rounded Tbs, or medicine cup works fine)
- 15 ml red watercolor paint

To make samples without Lyme/OspA
- 250 ml water
- 16 ml cornstarch (~1 slightly rounded Tbs)
- 15 ml red watercolor paint

To set up for the class
- bottle of white vinegar (for Anti-OspA)
- small Petri dishes (1.5 - 2 inch diameter)
- small dropper bottles for each group
- bathroom sized paper cups
- wax pencils
- plastic stirrers

Preparing “dog blood” samples

1. To make Lyme positive solution, combine skim milk with water. If you need more for your classes, keep the milk:water ratio the same at 1:3 (you will dilute the milk with 3 times the amount of water). Add cornstarch to mimic the Lyme negative solution as below. Mix thoroughly with stirrers.

2. To make Lyme negative solution, combine water with cornstarch and mix thoroughly using plastic stirrers. It is important to mix this solution completely to mimic the milk solution.

3. It is important to try to have both types of solution looking the same as possible so students will not be able to identify the solutions merely by observation. As a final step to create identical looking samples: Add several drops of paint to both solutions to make a colored liquid. Do not use too much paint. It will make the solutions too dark and the clumping will be difficult to see.

THE KEY IS TO CREATE SOLUTIONS ALIKE IN COLOR AND OPACITY SO STUDENTS CANNOT USE APPEARANCE TO DETERMINE WHICH SOLUTIONS ARE POSITIVE FOR LYME (OspA) AND WHICH ARE NOT.

4. As you create the final color, test the solutions along the way by removing a teaspoonful of each and adding 5 drops of vinegar to observe the level of reaction. When the
solutions are similar in color and the vinegar solution creates clumping in the Lyme solution, you are done.

WE HAVE OBSERVED A FEW FALSE NEGATIVES IN THE CLASSROOM—SAMPLES THAT SHOULD HAVE TESTED POSITIVE BUT DIDN'T. WE DISCOVERED THAT THIS IS DUE TO THE MILK SEPARATING FROM THE SOLUTION. THIS OCCURS WHEN THE SOLUTION IS LEFT STANDING EVEN FOR A FEW MINUTES BEFORE BEING POURED INTO CUPS FOR STUDENTS.

TO PREVENT SEPARATION YOU MUST KEEP STIRRING YOUR SOLUTIONS AS YOU POUR INDIVIDUAL STUDENT SAMPLES.

Setting up the lab

1. Create a set of 3-4 blood samples for each group using small dropper bottles. Each student group should have at least one positive Lyme sample, but it is preferable to have more. If you are concerned about having enough time for 3-4 samples per group, remember that each student in the group can be testing at the same time.

2. You may decide to number the cups in advance, or let the students do it as part of the testing procedure. If you prefer your student groups to select their own materials, you can allow each group to select the random samples for testing.

3. Create a blood sample for each group that is known to have Lyme disease. Label this sample “Lyme Positive” so students can use it as a control. They will test this first so they can identify the clumping reaction they find in the unknown blood samples.

4. Fill small dropper bottles with white vinegar and label as “Anti-OspA”. Have one bottle for each group.

5. To set up materials ahead of time, place the following on the tray or table for each group:
   - Blood samples in dropper bottles
   - Small Petri dishes (1 per sample)
   - Anti-OspA dropper bottle
   - Medicine cup
   - Wax pencil for labeling samples and/or test dishes

6. Remind your students to LIGHTLY shake ALL the bottles before testing. This is included in their instructions (see “Testing Blood for Outer Surface Proteins”, page 5). If the students shake too vigorously, it will create bubbles in the Lyme blood sample but not the non-Lyme samples.

   Once the Anti-Osp is added to the Petri dishes, make sure students swirl the solutions enough to produce a reaction. Poor mixing gives a weak reaction that can be confusing. On the other hand, shaking too vigorously can also mask a reaction.

   Students may think cornstarch residue is clumping. You can demonstrate the difference. The residue in the negative sample will disappear by gently swirling the dish. The visible particles in the positive sample will not dissolve back into the solution.
Lesson 2: Mix up with Osps

(There notes are added in parentheses to this copy of the Student Guide.)

Background

You are a microbiologist. Your team has been working on the spirochete, Borrelia burgdorferi. Scientists write this as B. burgdorferi. This is the bacterium that causes Lyme disease. You have been studying it to help make a test to diagnose Lyme disease in dogs.

You have found that B. burgdorferi has proteins on the outside. It is a common thing to find special proteins on the outer surface of any type of cell. In bacteria, these outer surface proteins are called Osps. They are part of the outer layer of the bacterial cell.

Osps are also antigens. Antigens cause our immune system to make antibodies. Antibodies are made to recognize and fight specific antigens. You can read more about this in the Discovery File: Immune System. Antibodies work by destroying the outer surface of bacteria. Antibodies can also make antigens to clump together. These actions will kill the bacteria.

(You may wish to have your students stop here and read the Discovery File on the Immune System as a class. This will help you to guide a brief discussion to include allergies, as many children are familiar with them. This real life experience can help them understand that outer surface proteins, Osps, are the same types of structures that cause them to itch or sneeze from an allergy. If your students are familiar with the concept of blood typing, you can explain that this, too, is based on outer surface proteins on red blood cells. We suggest that you reinforce using the term “Osp” in order to help students grasp this important concept.)

B. burgdorferi has many outer surface proteins. They are called OspA, OspB, OspC, OspD, and OspF. Different strains of B. burgdorferi can have different combinations of Osps. But you recently found something important!

You found that OspA is the only Osp found on every B. burgdorferi. You have decided to make a blood test for Lyme that looks for the OspA antigen. You can use an antibody solution called Anti-OspA. When Anti-OspA is added to OspA, it makes the OspA clump together.

(This can be a place to stop for class discussion. You can discuss how each antigen has a shape specific antibody—the lock and key model is useful for this.)

You have just been sent your first samples of blood from dogs that may have Lyme disease. You are excited about beginning to work on a test that will help veterinarians.

You decide to read over the steps for “Testing Blood for Outer Surface Proteins” (page 5) before you begin.
(Review the testing procedure together. Ask students why they think it is important that a control sample known to be positive for Lyme exists. This will allow you to see if they understand the general rationale behind using a control. It will also provide you with another opportunity to clarify or even illustrate the lock and key concept: Since Anti-OspA will only work against OspA, a clumping reaction to Anti-OspA proves the presence of OspA.)

Materials for the team:

- Set of containers with blood samples from dogs
- Container with known sample of blood positive for Lyme disease
- Dropper bottle labeled “Anti-OspA”
- Dropper
- Small Petri dishes—one for each blood sample
- Wax pencil
- Plastic medicine cup for measuring
- Paper cup with clean water

Discovery Files (background information): (Click on Discovery Files)
  - Tick Life Cycle
  - Bad Bull’s Eye!
  - Vaccines
  - Immune System
  - Immune System – Cell details

Procedure:
1. Work with your team. Decide how you will test the unknown Osp samples. Plan to use all of the materials.
2. Write down the steps you need to follow for your plan.
3. Make sure you include a control sample.
4. Carry out your plan and test all the samples.
5. Make a chart to show which blood samples (if any) have Lyme disease.
Testing Blood for Outer Surface Proteins — Clumping Reactions

All outer surface proteins (Osps) are antigens. Every Osp has a different shape from other Osps. Our bodies make a different antibody for each one. These antibodies have shapes that fit into each Osp exactly. They work like a lock and key.

Antibodies for a certain antigen are named by writing “Anti-” before the antigen they can fight against. For example, the antibody that fits OspF is called Anti-OspF. Anti-OspF will only fight OspF and not any other type of Osp. Every Anti-Osp antibody will make its matching Osp clump together.

To test for the presence of Lyme disease in a sample of blood:

- Place 5 ml. of the blood sample in a Petri dish. (5 ml = 1 tsp)
- Add 5 drops of Anti-Osp solution and swirl the dish gently on the table. You can move the dish in the shape of a figure 8. This will mix the contents well. Be careful that you don’t spill the sample.
- Look for signs of clumping.

What does clumping look like?

- Clumping looks like visible particles floating in the blood sample. This is a positive antigen-antibody reaction. It proves the presence of the Osp being tested for.
- If nothing happens to the sample, or if you simply see cloudy material at the bottom, this is a negative antigen-antibody reaction and proves the absence of the Osp being tested for.

NOTE: It is always a good idea to test a known blood sample first as a control. Follow the steps above and note what happens. Keep the clumped control sample. Use it to compare to other samples.
IMPORTANT FOR TEACHERS

Please make sure you THOROUGHLY RINSE OUT the dropper bottles immediately after final use! The cornstarch gets moldy if it is left in the bottles. Petri dishes also need to be washed, but they will not get moldy.

The bottles must then be cleaned—by hand in hot soapy water or as below.

BE BEST WAY TO WASH DROPPER BOTTLES:

- Remove tops from bottles and put spouts in upright position.
- Place the bottles in a “delicates mesh laundry bag,” zip shut and wash in the clothes-washing machine on the heavy-duty cycle for the longest time your machine will allow.
- Use WARM water.
- You may use COLOR-SAFE BLEACH.
- DO NOT USE FABRIC SOFTENER.
- DO use an EXTRA RINSE cycle if available.
- Do use an EXTRA SPIN cycle if available.
- Dry in clothes dryer on AIR ONLY cycle DO NOT USE HEAT CYCLE.
- Re-attach tops to bottles when dry (if still moist they will mold!).
- If there is any marker left, wipe off with clean dry cloth.