

Horseshoe Crab Blood Lab

Time Allowance:

60 minutes

Primary Goals:

- Simulating blood coagulation
- Simulating blood separation

What You'll Need: Materials required for ten (10) groups

- 1 graduated cylinder
- 1 mixing jar
- 30 pipettes (eye droppers)
- Safety glasses
- Nitrile safety gloves
- 1 container of white glitter
- 1 bottle white vinegar
- 1 bottle of Future® acrylic floor polish
- 1 small bottle of blue food coloring
- 1 small bottle of red food coloring
- 1 bottle mineral oil OR rheoscopic fluid with blue food coloring
- Water
- Ammonia (for cleaning up)
- 60 plastic vials
 - 10 labeled Human I
 - 10 labeled Human C
 - 10 labeled Horseshoe crab
 - 10 labeled Human I & horseshoe crab
 - 10 labeled Human C & horseshoe crab
 - 10 labeled Whole Horseshoe crab blood

In Advance: What you must do before class begins:

- "Human I" (infected)-- Measure 25 ml floor polish, 100 ml water and 1 ml red food dye in a mixing jar. (TIP: undiluted floor polish will give a more dramatic effect)
- Fill the 10 small vials labeled "Human I" with this mixture, and put the caps on the vials.
- Clean the mixing jar and graduated cylinder thoroughly.
- "Horseshoe crab" -- Measure 25 ml vinegar, 100 ml water and 1 ml blue food dye in a mixing jar. (TIP: undiluted vinegar will give a more dramatic effect)
- Fill the 10 small vials labeled "Horseshoe crab" with this mixture and put the caps on the vials.
- Clean the mixing jar and graduated cylinder thoroughly.
- "Human C"(uninfected) -- Measure 125 ml water and 1 ml red food dye in mixing jar.
- Fill the 10 small vials labeled "Human C" with this mixture and put the caps on the vials.
- Clean the mixing jar and graduated cylinder thoroughly.
- Fill the 10 small vials labeled "whole horseshoe crab blood" with mineral oil. Add white glitter. (If fabric paint is available add a few drops of blue paint NOTE: food coloring is water based and will not dissolve in the mineral oil.) Cap the vials.

Safety Tips - Explain to the class these tips for safety:

- Safety glasses are recommended. Although the materials being used in the experiment are generally safe, they can irritate or sting eyes upon contact. If contact does occur, flush eyes copiously with water.
- Safety gloves should also be worn during this experiment, since you will be simulating working with blood. It's important for students to understand that all health professionals wear gloves to prevent risks associated with contractible diseases like AIDS and hepatitis.
- Wash hands immediately after conducting the experiment. Do not put hands or fingers in mouth, or rub eyes, until after hands have been washed.
- Liquid materials used during the experiment can stain many surfaces, and should not be allowed to remain on clothing, upholstery or wood.
- Clean up any spilled materials immediately, as they can create a slip hazard on a tile or linoleum floor.

Demonstration- Explain to the class that they are about to investigate what happens when horseshoe crab blood is mixed with human blood.

Divide the class into 10 groups and arrange desks/chairs so that each member of the group can participate and observe the experiment.

Explain to the students that they are not working with actual blood samples -- that you have used special solutions of water, vinegar, floor polish and red food coloring to simulate real blood. **SAFETY CHECK!!!** Make sure all the students are wearing safety glasses and their gloves.

Part I - Distribute to each group:

1 vial of Human I "blood"	1 empty vial labeled "Human C and horseshoe crab"
1 vial of Human C "blood"	1 vial of "Whole horseshoe crab blood"
1 vial of Horseshoe crab "blood"	3 pipettes (eye droppers)
1 empty vial labeled "Human I and horseshoe crab"	Enough safety glasses and gloves for each student in the group

Instruct the students: Use one dropper for each of the blood types -- DO NOT mix the droppers.

Mixing Human clear and horseshoe crab blood.

- First, ask - what do think will happen when you mix blood from two different species?
- Carefully measure out a few dropperfuls of "Human C blood" and deposit it in the vial marked "Human C and horseshoe crab".
- Carefully measure out a few dropperfuls of "horseshoe crab blood" and deposit it in the same vial.
- Ask the students to report their observations aloud, record them in their workbooks. (Note to instructor, the mixture will not coagulate).

Mixing Human infected and horseshoe crab blood

- Carefully measure out a few dropperfuls of "Human I blood" and deposit it in the vial marked "Human I and horseshoe crab".
- Carefully measure out a few dropperfuls of "Horseshoe crab blood" and deposit it in the same vial.
- Ask the students to report their observations aloud, record them in their workbooks. (Note to instructor, the mixture will coagulate).

Discussion: Biomedical importance of the horseshoe crab

The horseshoe crab has led to tremendous advancements in eye research, development of surgical suture and wound dressings, and detection of bacterial contamination. Because of their large size, simple construction, and easy accessibility of the optic nerve the compound eyes of the horseshoe crab make the animal an ideal laboratory research subject. Medical researchers have been able to find out much of what we know about how our eyes function..

The shell of the horseshoe crab has also been extensively studied. Other arthropods have chitin as part of the chemical make-up of their shells but the chitin in the horseshoe crab shell is a very rare pure type. Chitin is a substance found in the shells, or exoskeletons, of horseshoe crabs, as well as other arthropods, such as lobsters, crabs, shrimps, spiders, beetles, and mosquitoes. It has received the attention of scientists because is non-toxic, biodegradable, and when processed to produce another substance called chitosan, can be used to produce a variety of important products.

Contact lenses, skin creams, and hair sprays can be made from chitin. It can be used to remove lead and other harmful metals that may be dissolved in drinking water, and clean certain harmful chemicals from wastewater. Chitin joins the fight against fat when it is added to foods. It has the ability to bind with fats and then passes them through and out of the body without being digested. Chitin can also be made into string used to sew up wounds and used in wound dressings. People do not have an allergic reaction to the stitches, which dissolve slowly, and the dressings actually promote healing. Many of these products are now available in Japan and are under development in the United States.

The most important discovery was that the blue, copper-based blood of the horseshoe crab contains a clotting agent that attaches to dangerous, fever-inducing toxins produced by many infectious bacteria. This clotting agent is called Limulus Amoebocyte Lysate, or LAL. The discovery of LAL yielded an excellent method of checking any drug for gram-negative bacteria, which are difficult to detect. Gram-negative bacteria are a group that causes a number of human diseases, including spinal meningitis. LAL is the standard test for injectable and intravenous drugs, required by the Food and Drug Administration (FDA).

Optional discussion: Blood Types - Before you begin, you **may** want to talk with the students about blood types.

In some ways, every person's blood is the same. But, when analyzed under a microscope, distinct differences are visible. In the early 20th century, an Austrian scientist named Karl Landsteiner classified blood according to those differences. He was awarded the Nobel Prize for his achievements.

Landsteiner observed two distinct chemical molecules present on the surface of the red blood cells. He labeled one molecule "A" and the other molecule "B." If the red blood cell had only "A" molecules on it, that blood was called type A. If the red blood cell had only "B" molecules on it, that blood was called type B. If the red blood cell had a mixture of both molecules, that blood was called type AB. If the red blood cell had neither molecule, that blood was called type O.

Why is blood type important?

There are times when a person might need to receive a blood transfusion -- that is, receive another person's blood to replenish his or her own. If someone is in a bad accident where they've lost blood, or if someone is having surgery or is ill, they may need blood to replace what they've lost and to keep the oxygen supply to the body at a healthy level.

It's important that an individual who receives a blood transfusion receive a blood type that is compatible with their own, or it could cause serious, life-threatening complications.

If two different blood types are mixed together, the blood cells may begin to clump together in the blood vessels, causing a potentially fatal situation. Therefore, it is important that blood types be matched before blood transfusions take place. In an emergency, type O blood can be given because it's most likely to be accepted by all blood types. However, there is still a risk involved. A person with type O blood is said to be a universal donor. A person with type AB blood is said to be a universal receiver. In general, however, it is still best to mix blood of matching types.

Part II – Separating Blood Cells from Plasma

Blood may be transfused as whole blood or as one of its components. Because patients seldom require all of the components of whole blood, it makes sense to transfuse only that portion needed by the patient for a specific condition or disease.

Whole blood is a living tissue that circulates through the heart, arteries, veins and capillaries carrying nourishment, electrolytes, hormones, vitamins, antibodies, heat and oxygen to the body's tissues. Whole blood contains red blood cells, white blood cells and platelets suspended in a fluid called plasma.

If blood is treated to prevent clotting and permitted to stand in a container, the red blood cells, weighing the most, will settle to the bottom; the plasma will stay on top; and the white blood cells and platelets will remain suspended between the plasma and the red blood cells. A centrifuge may be used to hasten this separation process.

Red blood cells contain hemoglobin, a complex iron-containing protein that carries oxygen throughout the body and gives blood its red color. Patients who benefit most from transfusions of red blood cells include those with chronic anemia resulting from kidney failure, malignancies, or gastrointestinal bleeding and those with acute blood loss resulting from trauma.

Plasma is the liquid portion of the blood -- a protein-salt solution in which red and white blood cells and platelets are suspended. It serves a variety of functions, from maintaining a satisfactory blood pressure and volume to supplying critical proteins for blood clotting and immunity. It also serves as the medium of exchange for vital minerals such as sodium and potassium, thus helping

maintain a proper balance in the body, which is critical to cell function. Plasma is obtained by separating the liquid portion of blood from the cells.

Cryoprecipitated AHF is the portion of plasma that is rich in certain clotting factors. It is used to prevent or control bleeding.

Platelets (or thrombocytes) are very small cellular components of blood that help the clotting process by sticking to the lining of blood vessels. Platelets are made in the bone marrow and survive in the circulatory system for an average of 9-10 days before being removed from the body by the spleen. The platelet is vital to life, because it helps prevent both massive blood loss resulting from trauma and blood vessel leakage that would otherwise occur in the course of normal, day-to-day activity. Units of platelets are prepared by using a centrifuge to separate the platelet-rich plasma from the donated unit of whole blood. The platelet-rich plasma is then centrifuged again to concentrate the platelets further.

Instruct the students to:

- Pick up the jar marked "whole blood" and shake it vigorously.
- Set the jar on a table, do not move it or pass it around.
- Explain that while the glitter is suspended in the mineral oil, it represents whole blood.
- As the glitter settles, explain that the solution represents separated blood -- the glitter representing "white blood cells" and the mineral oil representing plasma.

Discussion

What is a "centrifuge"?

A centrifuge is a powerful laboratory device that spins rapidly to magnify the force of gravity. It is used to separate blood samples into blood cells and plasma.

The centrifuge simulates an extreme force of gravity, which forces the blood to separate.

Doctors and other medical professionals use the separated blood for a variety of medical procedures.

- How do we preserve red blood cells and blood plasma?

(Whole and separated blood samples are preserved with special refrigeration and storage units.)

- Today, many individuals are not allowed to donate blood. Why?

(If an individual has an infectious disease, such as AIDS/ HIV or hepatitis, it can be fatal if it's administered to another individual through a transfusion. Individuals who have hyper/hypotension or an abnormal heart rate may also be denied permission to donate blood. Certain religious sects do not allow blood donation or transfusions. Hemophiliacs lack an important clotting agent in their blood, which could make blood donation fatal for them.)

Blood Chemistry – Lab Worksheet

Name: _____ Date: _____

Part One – Blood Coagulation

Materials needed:

- Safety glasses
- Safety gloves
- 3 pipettes (eye droppers)
- 3 labeled vials of “blood”
- 3 empty labeled vials
- 1 vial of “whole blood”

1. Define coagulation. _____
2. Prepare a mixture in the vial marked “Human C and horseshoe crab”
 - a. What do you think or expect to happen when you mix types “Human C” and “horseshoe crab”?
 - b. Carefully measure out several drops of “Human C” and put it in the vial labeled “Human C and horseshoe crab”.
 - c. Carefully measure out several drops of “horseshoe crab” and put it in the vial labeled “Human C and horseshoe crab”.
 - d. Record your observations:
3. Prepare a mixture in the vial marked “Human I and horseshoe crab”
 - a. What do you think or expect to happen when you mix Human I and horseshoe crab?
4. Which blood combinations are compatible? _____
5. Which blood combinations are not compatible? _____

Part Two – Whole Blood

1. Pick up the vial labeled “whole horseshoe crab blood” and shake it vigorously.
2. Set the vial on the table, do not move it or pass it around. Observe for three minutes.
3. Observations:
4. *On the back of this data sheet answer the following questions:*
 - a. Why did the “blood” separate?
 - b. Think of a way to separate the blood faster.
 - c. What is a centrifuge?