# HORSESHOE CRABS

## PREHISTORIC PARAMEDICS

**A film by Tom Fitz**

# Classroom Discussion Guide

## High School Version (Grades 9-12)

Content created for **Schoolyard Films, Inc.** by Cypress Curriculum Services, LLC

## Film Overview

When medicines are delivered directly into our blood, we have to be certain that they don't contain bacterial contaminants. So how do we know these medicines are safe? Believe it or not, the answer comes from a "living fossil," which still inhabits the mid-Atlantic coast of the USA—the amazing horseshoe crab. The latest film by award-winning filmmaker Tom Fitz, *Horseshoe Crabs: Prehistoric Paramedics*, tells the fascinating story of the Atlantic horseshoe crab and its vital connection to human health and shorebirds. This 15-minute educational film connects human health, environmental, and economic issues that are centered on the Atlantic horseshoe crab and its remarkable natural history.

Viewers will learn how this animal's unique immune system inspired scientists to develop an indispensible tool, which ensures injectable medications are free of bacterial contaminants. But as horseshoe crab harvesting increases to supply international demands for eel and conch bait, ecologists learn that not only humans rely on this intriguing animal. Migratory shorebirds like the threatened red knot, which feed heavily on horseshoe crab eggs during their northward migration, also declined in number as horseshoe crab harvesting pressure increased. Realizing the myriad values of the horseshoe crab, scientists, industries, and government agencies are now working together to maintain sustainable populations of the horseshoe crab.

## How to Use This Guide

*Horseshoe Crabs: Prehistoric Paramedics* serves as an excellent discussion starter and entry point for students to learn about this primitive arthropod, its interesting adaptations, and its importance to human health and the environment. This guide can be used to supplement study of the Next Generation Sunshine State Science Standards, specifically alongside lessons covering organization and development of living organisms, comparisons of functions of organs and other physical structures in animals, diversity and evolution of living organisms, and interdependence of living things. This guide also integrates Next Generation health education and language arts standards.

The "student briefing" provided on pages 3-8 may be distributed to the class prior to or after viewing the film and either read together or individually. The discussion questions on page 9 may be used as springboards to stimulate classroom discussion or as writing prompts. For an extension activity on the topic of arthropod molting, see the classroom activity on page 12.

## National Standards Correlations

| Discussion Guide Element | Unifying Concepts and Processes | Science as Inquiry | Science in Personal and Social Perspectives | Life Science | History and Nature of Science |
|---|---|---|---|---|---|
| Student Briefing | ● | ● | ● | ● | ● |
| Discussion Question #1 | ● | | | ● | |
| Discussion Question #2 | ● | | | ● | |
| Discussion Question #3 | | | | ● | |
| Discussion Question #4 | ● | | | ● | |
| Discussion Question #5 | | | ● | ● | |
| Discussion Question #6 | | | ● | ● | |
| Discussion Question #7 | | | ● | ● | |
| Discussion Question #8 | ● | | ● | ● | |
| Exploration Activity: LAL Gel-Clot Test | ● | ● | ● | ● | ● |

## Sunshine State Standards Correlations

| Discussion Guide Element | Next Generation Sunshine State Standards | | |
|---|---|---|---|
| Student Briefing | SC.912.L.14.2<br>SC.912.L.15.7<br>SC.912.L.17.6<br>SC.912.L.17.19 | SC.912.L.15.1<br>SC.912.L.17.2<br>SC.912.L.17.8<br>SC.912.L.17.20<br>CCSS.ELA-Literacy.WHST.9-10.1 | SC.912.L.15.4<br>SC.912.L.17.3<br>SC.912.L.17.11<br>CCSS.ELA-Literacy.WHST.11-12.1 |
| Discussion Question #1 | SC.912.L.15.1 | SC.912.L.15.4 | SC.912.L.15.5 |
| Discussion Question #2 | SC.912.L.15.1 | SC.912.L.15.4 | SC.912.L.15.5 |
| Discussion Question #3 | SC.912.L.17.11 | SC.912.L.17.12 | SC.912.L.17.13 |
| Discussion Question #4 | SC.912.L.17.1<br>SC.912.L.17.5 | SC.912.L.17.3<br>SC.912.L.17.6 | SC.912.L.17.8 |
| Discussion Question #5 | SC.912.L.17.1 | SC.912.L.17.5 | SC.912.N.4.1 |
| Discussion Question #6 | SC.912.L.14.52 | | |
| Discussion Question #7 | SC.912.N.4.1 | | |
| Discussion Question #8 | SC.912.L.14.6 | SC.912.L.14.52 | |
| Activity: LAL Gel-Clot Test | SC.912.L.14.2<br>SC.912.L.15.7<br>SC.912.L.17.6<br>SC.912.L.17.19 | SC.912.L.15.1<br>SC.912.L.17.2<br>SC.912.L.17.8<br>SC.912.L.17.20 | SC.912.L.15.4<br>SC.912.L.17.3<br>SC.912.L.17.11 |

# HORSESHOE CRABS

## PREHISTORIC PARAMEDICS

## Student Briefing

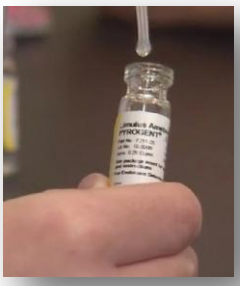
It's hard to look at a horseshoe crab without thinking of danger. Its spiny shell, ten spiderlike legs, and spear-shaped tail all appear threatening. But as forbidding as the horseshoe crab may appear, it is actually harmless. In fact, this amazing creature helps keep you out of harm's way. Each time you get an injection at a doctor's office, you can thank the horseshoe crab for protecting you from harmful bacteria. Read on to find out how!

### Living Fossils

Atlantic horseshoe crabs are a type of arthropod. Despite their name, horseshoe crabs are not actually crabs. They are more closely related to spiders and scorpions. But this evolutionary kinship with spiders and scorpions dates back hundreds of millions years. There are four living (extant) species of horseshoe crabs, and they very closely resemble their ancestors from long ago. In fact, the horseshoe crab has changed very little in its general appearance in over 400 million years. For this reason, it is often referred to as a "living fossil."


### Bacteria Beware!

So how do horseshoe crabs keep you safe from bacteria? The answer is in its unique immune system. Special cells in horseshoe crab blood can detect endotoxins, a harmful substance made by bacteria. When endotoxins are present, blood cells called amoebocytes release substances that cause the blood to form a gel. This immobilizes the bacteria and prevents them from spreading further into the body. The amoebocytes then release anti-bacterial compounds that kill the "captured" bacteria cells.



Humans may also be harmed if bacteria and their endotoxins enter our blood. It is very important that vaccines or other injected medicines are free of endotoxins. Even if the bacteria are killed through sterilization, the remaining toxins can be harmful. Fortunately, scientists figured out a way to use horseshoe crab amoebocytes to detect dangerous bacteria. The Limulus Amoebocyte Lysate (or LAL) test is now used around the world to make sure medicines are free of endotoxins.

Here is how the test generally works: A sample of the medicine is diluted in water. Several drops of this mixture are placed in a vial containing horseshoe crab amoebocytes, which were extracted from horseshoe crab blood. If the liquid mixture forms into a gel, then the medicine is contaminated with endotoxins from bacteria and cannot be used.

### FISH FOOD

Horseshoe crabs are not just important for human health. They are also important to the fishing industry. Horseshoe crabs are used as bait for eel and conch fishing. Harvesting horseshoe crabs for bait along the US Atlantic seaboard increased dramatically between 1970 and 1995. Faced with such pressures, horseshoe crab populations eventually began to decline.

### NOT ONLY HELPFUL TO HUMANS

Many shorebirds rely on horseshoe crab eggs for food. Each year, between April and June, horseshoe crabs emerge from the Atlantic Ocean to spawn on sandy beaches. Female horseshoe crabs bury thousands of eggs in the sand. Around this time, migratory shorebirds arrive from South America on their way to the Arctic for summer breeding season. The birds are hungry and the abundant horseshoe crab eggs help supply them with energy to finish the voyage. The red knot is one shorebird that really counts on horseshoe crab eggs. As horseshoe crabs declined due to over-harvesting or other population pressures, the number of nesting red knots in the Arctic also declined. Scientists hypothesize that there is a direct relationship between the number of horseshoe crabs and migration success of red knots.

Today, scientists, industries, and governments are working together to protect the horseshoe crab and the red knot. By placing a limit on the number of harvested horseshoe crabs, the population is growing. Horseshoe crabs are once again easy to spot on beaches and the red knots are returning.

## You might be WONDERING...

### Why is horseshoe crab blood **BLUE**?

Horseshoe crab blood contains a molecule called hemocyanin, which moves oxygen around the body. Each hemocyanin molecule contains two copper atoms. When oxygen is present, copper gives off a bluish hue. Human blood contains hemoglobin, which uses iron instead of copper to carry oxygen. Can you guess what color iron turns when oxygen is present?

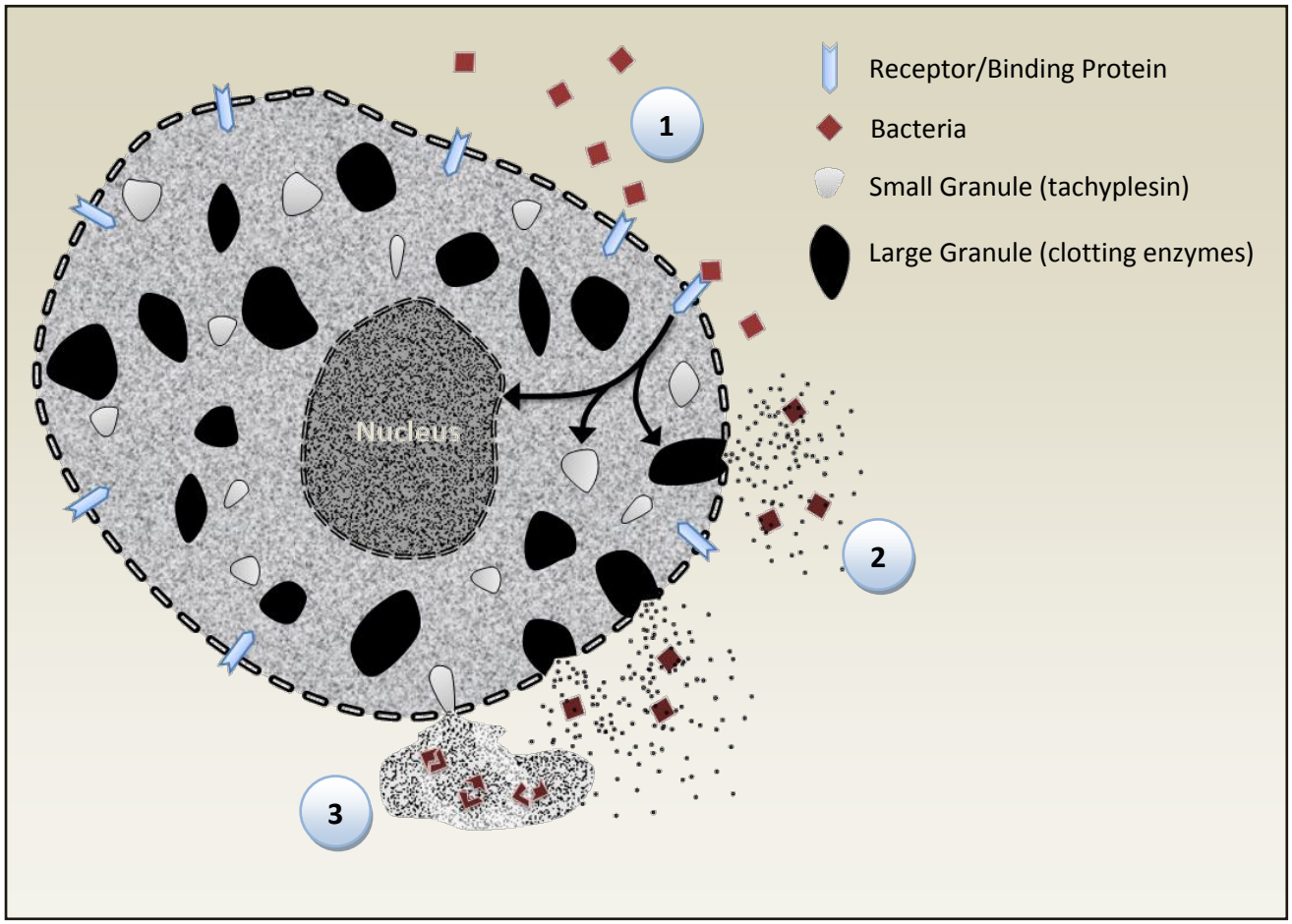
## A CLOSER LOOK

Horseshoe crabs and other invertebrates lack an adaptive immune system—they cannot develop antibodies to fight infections. Instead, the horseshoe crab has a blood-clotting mechanism that "traps" and inactivates harmful bacteria. The detection, immobilization, and destruction of bacteria in horseshoe blood follow three steps:

**1**—Receptors on the plasma membrane of the amoebocyte detect the presence of endotoxins from bacteria. The cell reacts by directing both small and large granules from within the cytoplasm to move toward the plasma membrane.

**2**—Clotting enzymes and coagulating proteins are released from the large granules into the surrounding plasma. This activates the clotting mechanism and a gel-like clot forms around the invading microbes.

**3**—Anti-microbial compounds, such as tachyplesin, are released from the small granules. These compounds destroy the bacteria.



Name: _____ Date: _____

HORSESHOE CRABS

PREHISTORIC PARAMEDICS

# Reinforce what you learned

**Multiple Choice:** Circle the letter that best answers the question or completes the sentence.

1. What do we call an animal that looks similar to related species that existed long ago?
   a. Dinosaur
   b. Living fossil
   c. Extinct
   d. Prehistoric

2. Hemocyanin transports which of the following elements through the blood for basic horseshoe metabolism?
   a. Copper
   b. Iron
   c. Protein
   d. Oxygen

3. What triggers an amoebocyte to release clotting and anti-microbial agents?
   a. Binding of endotoxins with specialized proteins in the plasma membrane
   b. Diffusion of bacteria into large granules
   c. Damage to membrane from endotoxins
   d. Signals from anti-microbrial compounds

4. The relationship between the red knot and the horseshoe crab is best described as—
   a. producer and consumer
   b. predator and parasite
   c. competitors
   d. predator and prey

5. If a LAL test sample forms a clot, which of the following must the sample contain?
   a. Iron
   b. Vaccine
   c. Hemoglobin
   d. Endotoxin

6. Which of the following terms does NOT go with the horseshoe crab?
   a. Arthropod
   b. Invertebrate
   c. Crustacean
   d. Exoskeleton

**Matching:** Write the letter of the description that best matches the numbered item in the blank provided.

____ 7. hemoglobin
____ 8. arthropod
____ 9. vaccine
____ 10. plasma
____ 11. antimicrobial
____ 12. plasma membrane

a. fluid containing blood cells
b. separates cytoplasm from plasma
c. usually has an exoskeleton
d. protein involved with oxygen transport
e. substance that kills or inhibits bacteria
f. increases immunity from certain diseases

Name: _____ Date: _____

HORSESHOE CRABS

PREHISTORIC PARAMEDICS

# In your own words

***At the end of the film, Dr. Al Segars contends that we must not only take care of the horseshoe crab, but also the area where they live. Explain what Dr. Segars meant by this statement.***

Write your explanation using at least two concepts from the film, the student handout, or your research. Use relevant facts, details, quotes, or examples to support your explanation. The provided word bank may be useful in developing your response.

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**Word Bank**

biodiversity

biomedical

conservation

consumer

diversity

economic

ecosystem

environment

extinction

fishery

habitat

impact

interdependence

predation

renewable

society

sustainability

wildlife

# HORSESHOE CRABS—PREHISTORIC PARAMEDICS

## Discussion Questions/Writing Prompts

*Use the following questions to stimulate classroom discussion or as writing prompts. Either way, the goal is to foster discussion on the level of synthesis and analysis. Below each question, you will find supporting information and recommendations to facilitate classroom discussion.*

1. **In the film we learned that very little has changed about the body design of horseshoe crabs in over 350 million years. How do we know this?**
   - Discuss how scientists use fossils and relative dating to understand evolutionary relationships of extinct species.
   - Explain that numerous species of horseshoe crabs appear in the fossil record over hundreds of millions of years but with very little change in physical appearance.
   - Explain that the term "living fossil" is often used to describe the horseshoe crab, but that it is not completely accurate. Technically, a living fossil is an extant species that also appears in the fossil record. The Atlantic horseshoe crab does not exist in the fossil record, but it is very similar to extinct species that do.
   - Give students other examples of "living fossils."
     - crocodiles
     - alligator snapping turtle
     - okapi
     - ginkgo tree

2. **What does the small change in horseshoe crab body design over hundreds of millions of years say about this species in terms of its ability to adapt to a changing environment?**
   - Ask students what usually happens to a species when it fails to adapt to changing environmental conditions.
   - Explain that many species demonstrate change in characteristics over time (evolution) but some species do not change or change very little.
   - Students should understand that the horseshoe crab appears to be very well adapted to the environmental conditions occurring over the last 350-450 million years.

3. **Scientists and citizens are trying to protect the horseshoe crab from overharvesting. Why should society care about the plight of horseshoe crabs?**
   - Horseshoe crab blood is a vital tool for making safe medicines.
   - The LAL test is the best test available for ensuring there are no bacteria or bacterial toxins in vaccines and other medicines.

- Ask students to consider the importance of horseshoe crabs to shorebirds like the red knot.
- Explain to students that over-harvesting for fishing can cause the horseshoe crab population to drop so much that future supplies of fishing bait may not be available.

**4. In addition to overharvesting, what are some other factors that may limit the size of horseshoe crab populations?**

- Possible responses include:
  - *Nesting habitat*—sandy beaches with low wave energy are important for successful spawning. Human disturbances of beaches can limit reproduction.
  - *Nursery habitat*—shallow bays with good water quality are important for young horseshoe crabs. Salinity is an important water quality factor. Juvenile horseshoe crabs prefer relatively high salinity.

  - *Predation*—as shorebirds and other egg predators increase in numbers, more horseshoe crabs will be eaten. Discuss the role of predation as a mechanism of population control. Explore the dynamics of populations in the context of predator-prey interactions.
  - *Food*—horseshoe crabs eat a variety of soft-shelled mollusks, small arthropods, and marine worms. Factors that result in declines of horseshoe crab prey would also result in declines of horseshoe crabs.

**5. The horseshoe crab monitoring program was discussed in the film. Scientists and volunteers attach a tag to a horseshoe crab. The tag has a unique ID number and a phone number to call if you find an animal with a tag.**

**a. If you found a horseshoe crab with one of these tags, would you take the time to report it? What information do you think would be most important to have when you call the number?**

- Tag identification number, the specific location (GPS coordinates best), date, time, whether the animal is alive or dead

**b. What are some of the ways this information helps scientists and government agencies?**

- Prompt students to recall from the film what knowledge is gained from tagging.
  - where the animals live
  - where they move
  - how long they live
  - the status of the population

- Remind students that government agencies must make regulations to protect a natural resource if it is threatened. Ask students how data from the tagging program might be beneficial to agencies tasked with making regulatory decisions.

6. **Why is it so important to keep bacteria out of vaccines and other medicines that are injected into your body?**
   - Discuss the role of bacteria and other germs in human sickness.
   - Point out that our skin and other surfaces (e.g. mouth, lungs, stomach) act as a barrier to germs.
     - mucus linings in sinuses and lungs
     - tears in eyes
     - acid in stomach
   - Discuss how all of these defenses are bypassed when something is injected directly into the bloodstream.
   - Ask students to identify another situation when bacteria may easily enter the body (i.e., cuts and skin abrasions)

7. **Prior to the development of the LAL test, health laboratories only used live rabbits to test whether injectable medicines were safe for human use. Rabbits received injections of the medicine and if they developed fevers, the medicine was determined to have endotoxins. In what ways do you think the LAL test is an improvement on the endotoxin test?**
   - Possible answers include:
     - more accurate
     - takes less time (90 seconds vs. several days)
     - more economical
     - less harm to animals (most horseshoe crabs are not harmed)

8. **Endotoxins are found in bacteria. Are there other infectious or toxic agents that we must keep out of injectable medicines? What are they?**
   - Possible answers include:
     - viruses
     - parasites
     - fungi
   - Discuss other methods commonly used to detect the presence of pathogens, such as antibody tests, cultures, DNA/RNA tests.

# Exploration Activity: LAL Gel-Clot Test

## TEACHER'S GUIDE

*In this lab, students will conduct the LAL gel-clot endotoxin test to reinforce their understanding of horseshoe crab amoebocytes and their use in medical laboratory testing. After viewing Horseshoe Crabs: Prehistoric Paramedics, students will use the LAL test to determine whether pond water contains endotoxins from gram-negative bacteria. While samples are incubating, the teacher will present a brief slideshow about endotoxins, the horseshoe crab immune system, amoebocyte structure and function, and the LAL test.*

*This activity is adapted from the LAL-LAB: BACTERIA, BLOOD & BIOMEDICAL TESTING activity originally developed by the Delaware Division of Fish and Wildlife, Aquatic Resources Education Center.*

### Class Time

**Film Viewing:** 15 minutes

**LAL Test:** 15 minutes

**Presentation:** 10 minutes

**LAL Test Interpretation:** 15 minutes

### Materials

- Distilled water (approx. 250 ml per student or student team)
- Pond water (or other biologically contaminated water source)
- Computer and projector

Each student will need the following:

- 2 gel-clot LAL single test vials
- 2 1-ml graduated individually-wrapped sterile plastic pipettes
- 2 sterile cups
- Distilled water
- Pond or aquarium water
- labeling tape & marking pen
- Student instruction sheet
- Student worksheet

Note: If time or supplies are limited, students may work in teams or the test can be done as a demonstration by the teacher.

Sources of the LAL tests are provided at the end of this document.

**Background Information**

- Bacteria are single-celled organisms found everywhere, including inside our bodies.
- Humans benefit from certain bacteria in many ways—nitrogen fixation, decomposition, food digestion, fermentation of foods, biotechnology applications. Other types of bacteria are harmful to humans and may cause serious infections.
- *Gram-negative bacteria* refers to a broad category of bacteria that are characterized by having an outer membrane containing substances called *endotoxins*.
- Endotoxins are a type of *pyrogen*. When pyrogens enter the body they cause fevers. In serious cases, endotoxins can cause comas and even death.
- Our skin acts as a barrier to prevent bacteria from entering our bodies. We are not harmed by endotoxins when we swallow them because our digestive system breaks them down.
- When endotoxins are put directly into our bloodstream, the body's barrier defenses are bypassed and we can get very sick.
- Sterilization helps remove living gram-negative bacteria, but not endotoxins-- they can persist in the solution even after heat sterilization.
- Any medicine or medical equipment that comes into direct contact with blood or internal tissue must be free of endotoxins. This includes vaccines, injectable medications, medical implants, needles, surgical tools.
- Horseshoe crabs live in a marine world teeming with gram-negative bacteria, but they lack an adaptive immune system—they cannot develop antibodies to fight infections as humans can.
- Horseshoe crabs have a blood clotting mechanism involving specialized cells called amoebocytes. The clotting mechanism involves detection, immobilization, and destruction of gram-negative bacteria.
- Receptors on the plasma membrane of the amoebocyte detect the presence of endotoxins from bacteria. The cell reacts by directing small and large granules located within the cytoplasm to move toward the plasma membrane.
- Clotting enzymes and coagulating proteins are released from the large granules into the surrounding plasma. This activates the clotting mechanism and a gel-like clot forms around the invading microbes.
- Anti-microbial compounds, such as tachyplesin, are released from the small granules and destroy the bacteria.
- The clotting component of this immune response is the basis for the Limulus Amoebocyte Lysate (LAL) test.
- Frederick Bang, a medical researcher, first identified the mechanisms of the horseshoe crab immune response in a 1957 research paper. He later collaborated with Dr. Jack Levin, a hematologist (blood specialist), to pioneer the methods of the LAL test.

- After Bang and Levin published their findings, other researchers investigated and refined the LAL technology. Today, the LAL test is an industry standard for detecting endotoxins in certain medicines and medical equipment. Many lives have been saved by this scientific discovery.
- Horseshoe crabs play a surprising role in human wellbeing. This is another example of how the Earth's plants and animals can be very important to society and why we should work to protect as much biological diversity as possible.

**Procedure**

***Pre-class Preparation***

- Success of this demonstration depends on access to endotoxin-free (pyrogen-free) water for use as a control. Since contaminated distilled water may contain enough bacteria or endotoxins to give a positive test result, it is critical that you test distilled water prior to the lab. Using the procedures described below, test the distilled water to confirm that it is endotoxin-free.
- Collect "contaminated" water from a pond, aquarium, or similar source.
- Gather and set out other required materials. Set up computer and projector for film viewing and presentation. Have distilled and contaminated water available in vessels that students can pour from into their sample cups.

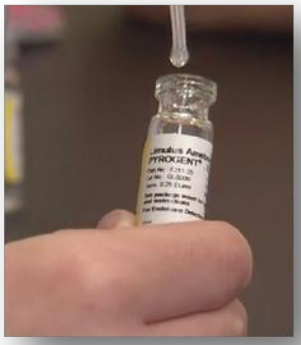
***Class Introduction***

- After viewing *Horseshoe Crabs: Prehistoric Paramedics*, explain to the class that they will now conduct their own LAL gel clot test.
- Show the class an LAL gel-clot vial. Ask the students what they think the vial contains. Students should recall from the film that the vial contains components of horseshoe crab blood, specifically amoebocytes. Explain to students that LAL stands for *Limulus Amoebocyte Lysate*. *Limulus* is the genus of the horseshoe crab, *amoebocyte* references the specialized cells involved in the test, and *lysate* refers to the contents of the amoebocytes that are collected during the extraction procedure (isolated amoebocytes are placed in purified water, which causes them to lyse. The cell contents, or lysate, are then isolated, freeze dried, and placed into test vials).

***LAL Gel Clot Test***

- Pass out the student instruction sheet provided below, then review the procedure. Students should wash their hands thoroughly before and after this laboratory activity.
- Once the students have completed the test and cleaned up their lab space, show the class the supplementary presentation while the samples incubate.
- Once the incubation period has ended, ask students to examine their sample vials and complete the worksheet provided below. Alternatively, the questions provided in the worksheet may be reviewed and discussed as a class.

## Student Instructions: LAL Gel-Clot Test



In this activity you will test for the presence of endotoxins using the same LAL test used by researchers and medical companies. You will test two samples—distilled water and pond water. After conducting the tests, you will complete a worksheet on your observations and answer follow up questions.

**Step 1:** Gather and organize materials. Each student (or team of students) needs the following:

- 2 gel-clot LAL single test vials
- 2 sterile plastic graduated pipettes (1.0 ml)
- 2 sterile cups
- Sterile water
- Pond (or aquarium) water
- Permanent marker

**Step 2:** Set up sample cups and gel clot vials.

- Label one cup “SW” (i.e. sterile water) and the other “PW” (i.e. pond water). Take care not to touch the inside of the cups.
- Pour approximately 20 ml of purified water into the cup labeled “SW.” Pour approximately 20 ml of the pond water into the cup labeled “PW.”
- Label one of the LAL gel clot vials *"SW"* and the other *"PW."*

**Step 3:** Collect and incubate samples

- Remove the first pipette from its sterile wrapping. Identify the 0.25 ml mark on the pipette. You may want to mark the 0.25 ml level with a permanent marker if it is difficult to see.
- Remove the seal and cap from the vial labeled "*SW*." Hold the vial cap or place it on a clean surface. Also be careful not to touch the vial opening.
- Squeeze the pipette bulb then insert the tip into the sterile water sample. Slowly release the pressure with your fingertips until the sample partially fills the pipette. Now apply pressure to the bulb until the sample reaches the 0.25 ml level. Keeping steady pressure on the bulb, move the pipette over the vial. Slowly apply pressure until all the sample is transferred into the vial.
- Immediately recap the vial, and then swirl the contents several times to mix.
- Using the second pipette, repeat these steps for using the cup and vial labeled *"PW."* Set both vials to the side. They will need to incubate for 15 minutes. Clean up and put away materials as instructed by your teacher.

**Step 4:** Observe test results

- At the end of the incubation period, pick up each vial and invert it.
- Compare the samples in each vial and enter your observations on the student worksheet.

Name: _____ Date: _____

HORSESHOE CRABS
PREHISTORIC PARAMEDICS

# LAL TEST: STUDENT WORKSHEET (page 1 of 2)

## Test Results

| Sample ID | Sample Description | Did a gel clot form? Yes or No? | Observations |
|---|---|---|---|
| | | | |
| | | | |

1) What is the purpose of testing sterile water in this test?

_____

2) What can you conclude from this test? Explain your answer.

_____

_____

_____

3) In your test, the sterile water sample may have formed a clot. What are two possible explanations for this sample forming the gel clot?

_____

_____

# LAL TEST: STUDENT WORKSHEET (page 2 of 2)

Use the word bank to complete the following statements.

1) Endotoxins are formed on the cell membrane of _____bacteria.
2) The _____ reaction in the LAL test confirms the presence of _____
3) A positive result in the LAL test does not necessarily confirm the presence of living _____.
4) The LAL test is NOT an appropriate test for detecting the presence of _____.
5) The purpose of a _____ in the LAL test is to verify that a positive result (clot formation) is not due to contamination during the test.
6) Endotoxins are a type of _____ that causes fevers when in the body of mammals.
7) _____ found on the cell membranes of _____ are able to detect the presence of bacterial endotoxins.
8) _____ is the genus name for the American horseshoe crab.
9) The large granules inside amoebocytes contain _____.
10) The horseshoe crab immune system does not produce _____.

**Word Bank**

amoebocytes
antibodies
bacteria
clotting enzymes
control
endotoxins
gel-clot
gram-negative
limulus
pyrogen
receptors
viruses

**TEACHER KEY**

## LAL TEST: STUDENT WORKSHEET (page 1 of 2)

Test Results

| Sample ID | Sample Description | Did a gel clot form? Yes or No? | Observations |
|---|---|---|---|
| | | | |
| | | | |

1) What is the purpose of testing sterile water in this test?
*Sterile water is a test control. It is used to confirm that a positive result is not due to contamination.*

2) What can you conclude from this test? Explain your answer.
*If the test was negative for the control and positive for pond water, the student should conclude that endotoxins were present in the pond water but not in the sterile water. Students may recognize that the LAL test is not confirmation of living bacteria, only endotoxins.*

3) In your test, the sterile water sample may have formed a clot. What are two possible explanations for this sample forming the gel clot?
*A positive result in the sterile water sample confirms that the sample contained endotoxins. The endotoxins could have been present in the distilled water or may have entered the sample as a result of contamination during the test (e.g. transfer from students hands) or from contaminated testing equipment.*

**TEACHER KEY**

# LAL TEST: STUDENT WORKSHEET



Use the word bank to complete the following statements.

1) Endotoxins are formed on the cell membrane of *gram-negative* bacteria.
2) The *gel-clot* reaction in the LAL test confirms the presence of *endotoxins*.
3) A positive result in the LAL test does not necessarily confirm the presence of living *bacteria*.
4) The LAL test is not an appropriate test for detecting the presence of *viruses*.
5) The purpose of a *control* in the LAL test is to verify that a positive result (clot formation) is not due to contamination during the test.
6) Endotoxins are a type of *pyrogen* that causes fevers when in the body of mammals.
7) *Receptors* found on the cell membranes of *amoebocytes* are able to detect the presence of bacterial endotoxins.
8) *Limulus* is the genus name for the American horseshoe crab.
9) The large granules inside amoebocytes contain *clotting enzymes*.
10) The horseshoe crab immune system does not produce *antibodies*.

**Word Bank**

*amoebocytes*
*antibodies*
*bacteria*
*clotting enzymes*
*control*
*endotoxins*
*gel-clot*
*gram-negative*
*limulus*
*pyrogen*
*receptors*
*viruses*

## Sources for LAL Single Gel-Clot Tests

**Lonza Group Ltd.**

www.lonza.com

Customer Service:

(800) 638-8174

Scientific Support:

(800) 521-0390

**Charles River Laboratories International, Inc.**

www.criver.com

Product & Service Inquiries:

(877) 274-8371