



charles river

Atlantic Horseshoe Crab Conservation

The Atlantic Horseshoe Crab

The Ancient Mariner

The Atlantic horseshoe crab is an ancient mariner with a lineage that dates back 440 million years. Though they look similar to crustaceans, they actually belong to the subphylum Chelicerata, relating them more closely to spiders and scorpions. *Limulus polyphemus* is found along the Atlantic and Gulf coasts from northern Maine to the Yucatán Peninsula, with the Delaware Bay as the center of the population. This interesting animal is important not only for the pivotal role it plays in its ecosystems, but for its valuable contribution to the field of biomedical research and its applications.



Left, an Atlantic horseshoe crab arriving on the beach to spawn.
Center, spawning season for the Atlantic horse shoe crab.
Right, horseshoe crab being cleaned in a water bath before blood collection.



Atlantic horseshoe crabs during spawning season.

The Atlantic Horseshoe Crab

A Living Fossil.

As keepers of the environment, we have a responsibility to preserve, protect, and live harmoniously with the animals we share the planet with. With its sturdy helmet-like shell and intimidating pointed “tail,” the Atlantic horseshoe crab looks perfectly capable of protecting itself. This is true to an extent, but this amazing creature’s important contribution to our everyday health and safety grants it special attention.

As a source of critical proteins that detect the presence of endotoxins in products and devices used by humans, the Atlantic horseshoe crab has long been treasured by the biomedical industry. For this reason, it is important that we serve as advocates for the humane treatment of these animals, and strive to achieve balance between our need for this valuable material and the livelihood of the animal that provides it. Charles River is proud to play a role in alleviating pressures on horseshoe crab populations through tireless [conservation efforts](#), active animal welfare campaigns, and decades of research and development.



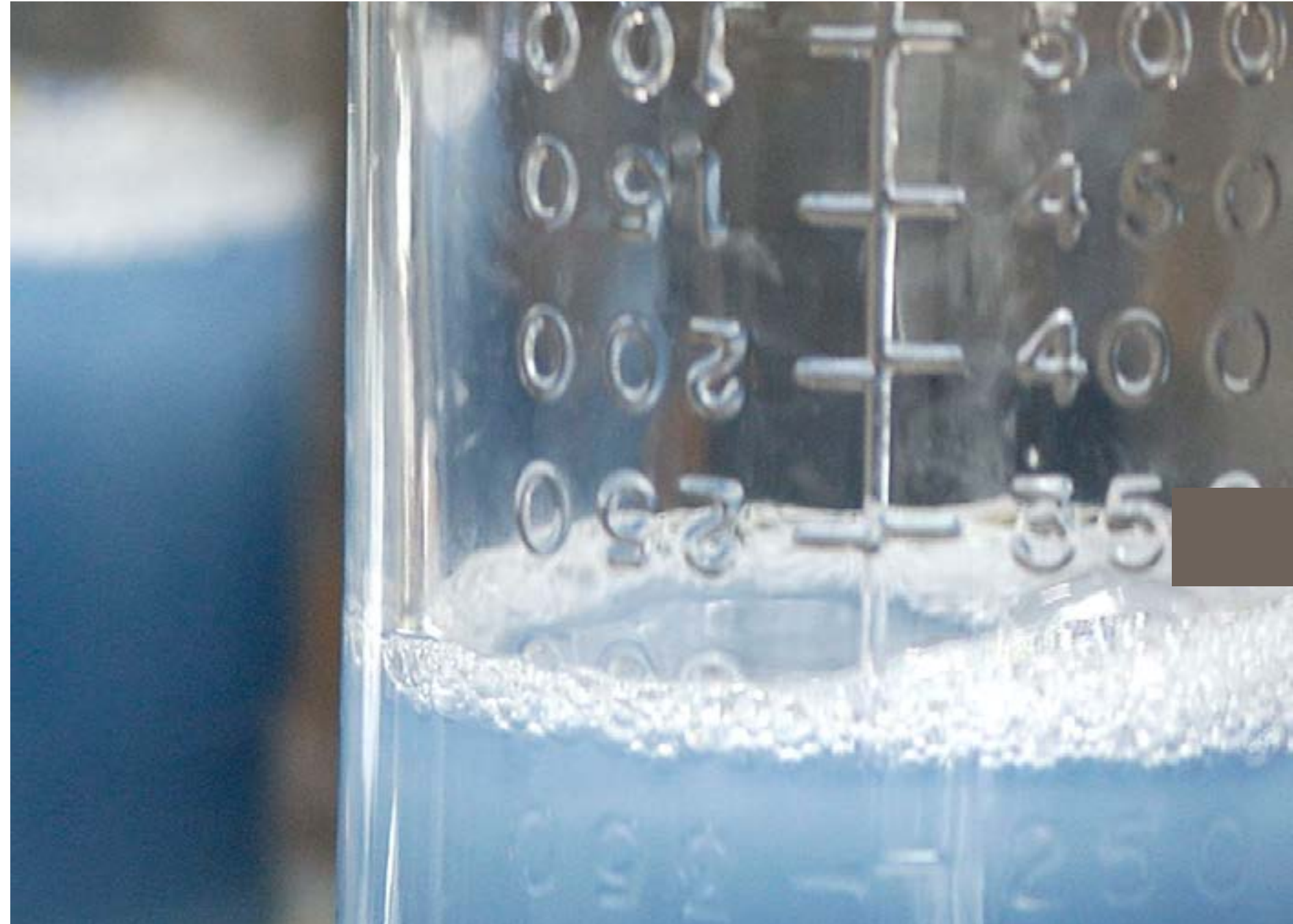
Fossilized remains of a Horseshoe Crab.

Anatomy

We Bleed Blue.

The simplicity of this ancient creature's exterior is a dramatic contrast to the complexity of its mysterious blood. Unlike the blood of vertebrates that uses hemoglobin to carry oxygen, the blood of the horseshoe crab instead uses copper-containing hemocyanin, which turns the animal's blood a striking shade of blue.

The true beauty of this remarkable creature, however, is seen in its primitive immune system. Instead of developing antibodies in response to infection, the immune system releases proteins that can bind and kill bacteria. In addition, other immune proteins clot when exposed to waterborne bacteria, a mechanism which forms the basis of the [LAL test](#).



Blood collection from an Atlantic horseshoe crab.

Horseshoe Crab Reproduction

Born to Save.

During spring and early summer, nesting females will make several trips up the beach to spawn. With each trip, she will dig a small nest and lay a series of four to five discrete egg clusters. These clusters are located in dense masses near the front of her shell, and typically contain 4,000 eggs per cluster. By the end of the season, she will have deposited as many as 20 clusters. As an important source of food for shore birds, many of these thousands of eggs will not live past the larval stage. Survivors hatch at two weeks and move into shallow waters where they will live for a year or more, moving deeper into the ocean as they mature.



Left, Atlantic coast during horseshoe crab spawning season.

Center, a cluster of horseshoe crabs on the Atlantic coast.

Right, licensed fisherman hand-collecting Atlantic horseshoe crabs.

Limulus Amebocyte Lysate

Exquisitely Exact.

Limulus amebocyte lysate (LAL) is an aqueous extract of blood cells (amebocytes) from the horseshoe crab. Comprised of proteins, LAL is used to detect the presence of endotoxins. In the **50 years** since this discovery, the blood of the horseshoe crab has served as a natural indicator of bacterial contamination in critical medical treatments all over the world.

[So why is endotoxin detection so important?](#) Endotoxins are a cell wall component of Gram-negative bacteria that cause a pyrogenic response (fever). They are extremely potent, heat stable, and present everywhere bacteria are or have been. With its specificity, simplicity, and remarkable sensitivity, LAL ensures the safety of products such as intravenous drugs, vaccines, and medical devices.

With more than 80 million endotoxin tests performed annually for injectable pharmaceutical products and implantable medical devices, there has never been a single FDA-confirmed pyrogenic outbreak due to a false-negative LAL result.

Bacterial Endotoxin Testing

Safe & Sustainable.

Years of research and application have proven LAL to be unequivocally recognized as the most sensitive method available for the detection of endotoxins. Approximately **80 million** LAL tests are performed each year, and there have been no FDA-confirmed pyrogenic outbreaks due to the failure of LAL to detect the presence of endotoxin. As a highly regulated product, manufacturers of LAL undergo biannual inspections by the FDA and must conform to CGMP requirements. Of course, to manufacture LAL, the blood of the horseshoe crab must be carefully collected through a controlled bleeding program. Policies such as hand-collecting and same-day return to the sea ensure the animal's safety. [With a focus on innovation, humane care, and environmental protection](#), Charles River has responsibly manufactured LAL since 1987.

Prior to FDA approval of LAL in the 1980s, the rabbit pyrogen test was the standard FDA-approved test for endotoxins. This test, however, was labor intensive, lengthy, expensive, and rabbits perished unnecessarily. By contrast, the blood of the horseshoe crab is carefully collected through a controlled breeding program, hand-collecting, the horseshoe crab is returned to sea the same day.

To illustrate, LAL can detect as little as 0.5 pg/mL of harmful bacterial endotoxin (equivalent to one grain of sand in an Olympic-sized pool).



Endosafe® Nexus™ Automated Endotoxin Testing System utilizing cartridge technology.

Our Collect and Release Method

Hand-collected.

We are committed to employing only fishers licensed by the Department of Natural Resources to hand-collect (as opposed to trawling) horseshoe crabs from the coastal waters of South Carolina.

Our highly controlled and monitored procedures enable us to collect enough raw material for 24 months of LAL production from a minimum number of donor animals. Today, the horseshoe crab's blood is still [the most specific, accurate, and reliable detector](#) of dangerous bacterial endotoxins.

In South Carolina, horseshoe crabs must be:

Collected by hand

**Used only for biomedical applications
(LAL production) and marine
biological research**



Licensed fisherman hand-collecting Atlantic horseshoe crabs.

LAL Production

Humanely Produced.

1: Once the hand-collected crabs¹ are brought into our lab, they are carefully inspected by trained employees following humane care practices who determine the animal's health and maturity. At the facility, crabs are cleaned in a waterbath², inspected, and prepped for bleeding. About **25%** of the crab's blood is removed in the collection of their blood cells, or amebocytes³.

2: After careful collection, the crabs are quickly returned unharmed to their native waters within 12 hours, and their blood volume rebounds quickly.

3: The amebocytes are prepared and treated according to Charles River's proprietary formulation process to yield our final product⁴.

4: The LAL is tested and analyzed for performance characteristics according to our FDA license.

5: The finished [LAL product](#) is used in quality control laboratories within industry companies to ensure their medical products are free of contaminating endotoxin⁵.



1



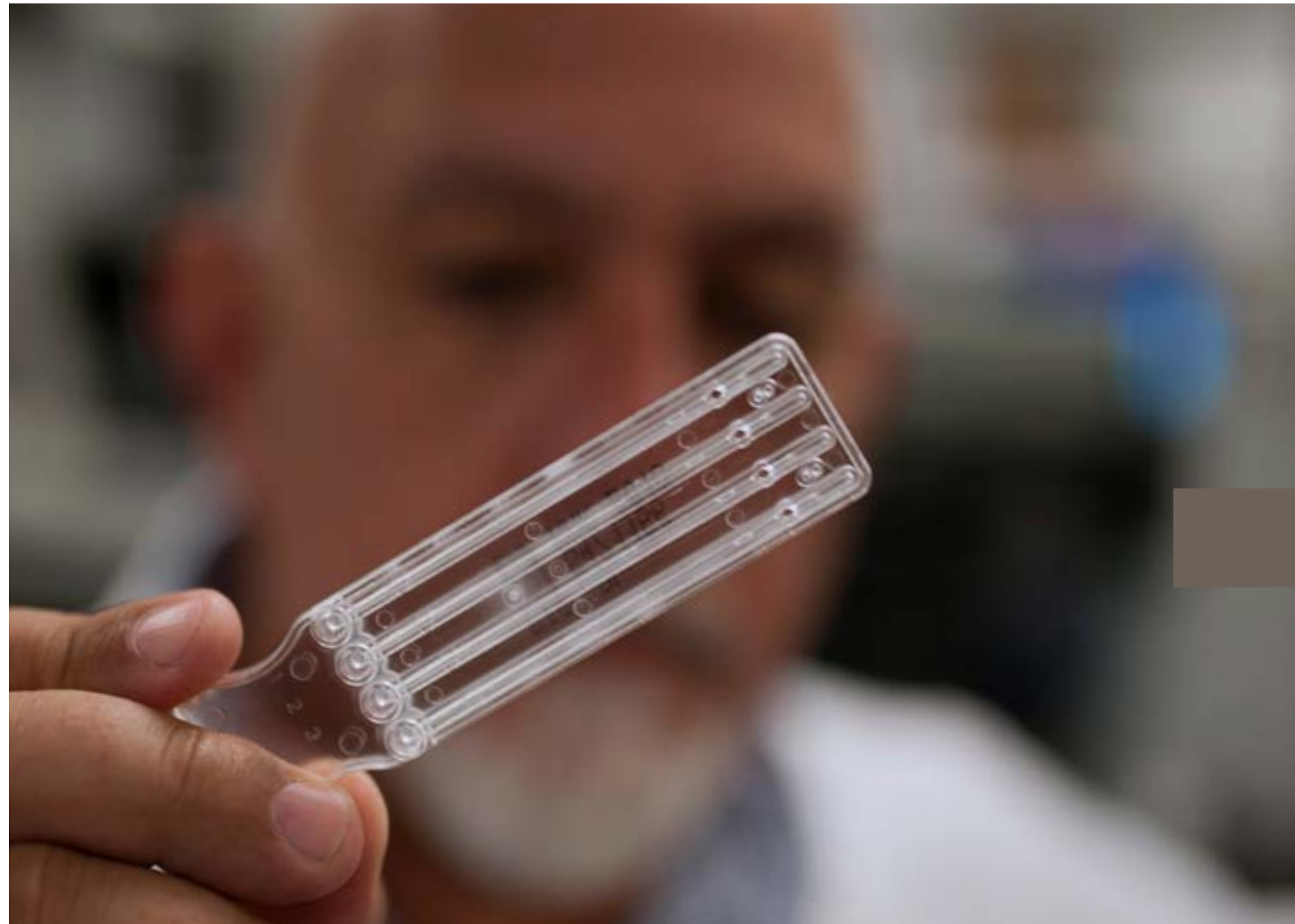
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3



4



The Impact of the 3R's Imperative on Endotoxin Testing

Replace, Reduce, Refine.

The [3Rs](#) have been influencing endotoxin testing methods for over **60 years**. The goal is to replace, reduce, or refine any test that uses an animal model as an *in vivo* assay. These are the fundamental principles that drive ethical research, testing, and education using animals. With a focus on increasing corporate responsibility and maintaining a progressive ethical approach to animal welfare initiatives, many organizations are looking to non-animal-derived endotoxin testing methods. It is now more important than ever that we reaffirm our commitment to and compliance with the 3Rs imperative, as these principles are being incorporated into legislation, guidelines, and practice.

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Underside of an Atlantic horseshoe crab.

Charles River Advocacy

Working Thoughtfully.

Many companies think that moving away from LAL to reduce the use of the horseshoe crab will help them comply with 3R principles. This idea should be carefully evaluated, as moving to recombinant alternatives could produce counterproductive effects by endangering the [conservation](#) efforts Charles River and others have fought to implement.

We believe the adoption of recombinant technology as an alternative to LAL must be based on substantial and incontrovertible data where equivalency can be proven. Any new technology should be released only when we are certain that it is equal to or better than LAL. Contrary to popular belief, misleading media coverage, and unrelenting bias, the biomedical industry is not the horseshoe crab's biggest threat. In fact, the industry has driven the development of laws to protect the animal against its biggest threats; commercial bait fishing, coastal development (and resulting loss of historical spawning sites), and sea level and climate change rank among the top threats to the species population.



Charles River technician with Atlantic horseshoe crab blood used to make LAL.

Involvement with Government Regulations

Protected & Respected.

The horseshoe crab's survival can be attributed in part to current government protection. Previously, fishing industry demands for *Limulus polyphemus* made it necessary for South Carolina to develop a state-wide conservation plan. In 1992, Dr. James Cooper, a pioneer in the research and development of the LAL assay, wrote draft legislation calling for the management and regulation of horseshoe crab fisheries. As a result, the state enacted laws protecting the indigenous horseshoe crab population. Now, with the addition of six island sanctuaries, the horseshoe crabs in South Carolina are one of the most protected species on the East Coast of the United States.

Without the need for LAL in biomedical use, the legal protection of the horseshoe crab is not guaranteed, and they would again fall prey to overfishing and use as bait. It is our shared responsibility to be diligent with an eye to the bigger picture moving toward new solutions while ensuring animal welfare, patient safety, and regulatory compliance are the utmost priority.

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Horseshoe crabs being hand-collected to help produce LAL for endotoxin testing.

The State of the Population

Upward Trend.

According to the 2019 Horseshoe Crab Benchmark Stock Assessment Peer Review Report published by the Atlantic States Marine Fisheries Commission (ASMFC), the population continues to remain stable where the biomedical industry is present in the Northeast and thrive and grow in the Southeast due to protection efforts – a trend spanning decades. Additionally, the Southeast Area Monitoring & Assessment Program (SEAMAP) Coastal Trawl Survey, conducted by the South Carolina Department of Natural Resources, shows a population increase over the last several years. Two results from a South Carolina tagging study also demonstrated that bled horseshoe crabs are able to return to spawning beaches in subsequent years.

Whether you're a drug manufacturer, a health care professional, or an animal lover, we invite you to help raise awareness about these amazing creatures, the role they play in our everyday lives, and what we can do to protect them.

For more information about our commitment to the conservation of the Atlantic horseshoe crab, related resources, and the references cited in this brochure, please visit us at www.criver.com/hsc.

Email your questions to hsc@crl.com

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Atlantic horseshoe crabs during spawning season.




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Endosafe® distribution, sales and service locations,
LAL reagent manufacturing only in Charleston, SC

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