

AN INTERVIEW WITH...

Brett Hoffmeister

LAL Production Manager
Associates of Cape Cod, Inc.

Chair to the Horseshoe Crab Advisory Panel
Atlantic States Marine Fisheries Commission



Specialists in Endotoxin and Glucan Detection

Can you give us a quick overview of your own background and also about Associates of Cape Cod, Inc. (ACC)?

Brett: Absolutely. Associates of Cape Cod has been located in Falmouth, Massachusetts since the beginning, since we were founded back in the '70s. The founder of our company, Stanley Watson commercialized LAL. We were the first to bring it to the market and we've been growing here since. It's been a great run for ACC. I started with the company in 2003. I've got a background in manufacturing. I love the ocean, I've been a resident of Cape Cod for longer than I care to admit, over 30 years. We found each other and I've been working with horseshoe crabs ever since. I'm really enjoying my experience here and the things I've learned.

Why is it important to test for endotoxins?

Brett: Endotoxins are everywhere. We're surrounded by them. They're a component of our life, but they're dangerous when they enter your bloodstream or spinal fluid. Endotoxins that you and I are exposed to on a daily basis, our bodies can handle, but if it does cross that bloodline, it becomes a problem. It's pyrogenic, it can cause a fever. It can cause terrible things to happen to the human body, up to and including death. Endotoxins are something that need to be tested for in anything that's injected or implanted into a human body.

Why are horseshoe crabs so important for endotoxin testing?

Brett: Horseshoe crabs have a component of their blood that clots around the presence of endotoxins. It's part of their immune system. Other animals have it but horseshoe crabs are readily accessible. It's a very primitive and simple mechanism to manipulate. They provide the LAL manufacturers in the United States, the raw material to produce the product that we make that test endotoxin. Essentially, we take those components from the blood, manipulate them and provide an assay in a test tube that's freeze-dried, it's stable and it's very accurate. It's very cost-effective.

Are there other ways to test for endotoxins as well?

Brett: There are. Prior to the '70s, the rabbit pyrogen test was the go-to test where pharmaceutical companies would inject a rabbit with, say, a sample flu vaccine for instance. They have to monitor the rabbit for two or three days to see if it spiked a fever. That test is still around today. It's not widely used, but it's a compendial test. It still exists. There's an MAT test that's based on human blood.

Most recently, there's a lot of news about RFC tests for Recombinant Factor C. Most recently ACCs actually released a new product, an rCR, a Recombinant Cascade Reagent, which more closely mimics the test based on the LAL test, so the same cascade. There are other tests out there, but the gold standard, the compendial test right now remains the LAL.

What does LAL stand for and what does it actually mean?

Brett: That's a great question. LAL stands for Limulus Amoebocyte Lysate. A lot of people say that crabs contain LAL and it's not the truth. The Limulus is based on the Limulus polyphemus, the American horseshoe crab. The lysate that we produce is based off of the amoebocyte. We lyse the amoebocytes and release those clotting agents that I was talking about. This is a product that we make from the blood of the horseshoe crabs.

If you're using horseshoe crabs for the testing, that must have an impact on the population, doesn't it, or does it not have any impact when you're using it for biomedical use?

Brett: That's a great question. There's a lot of debate about that. There's a lot of data, there's scientific data, volumes of scientific data that'll tell you that the impact is really minimal with what we do with the horseshoe crabs. In the United States, the primary use of horseshoe crabs is for bait. They use it to catch carnivorous snails or conch. The biomedical use is a much smaller component of that entire fishery. The population in the United States is healthy enough to withstand a bait harvest, and the biomedical mortality is a fraction of that bait harvest. It impacts a fraction of the population of horseshoe crabs that's measured in the tens of millions up and down the East Coast.

What do you say to people then who say that the population in the US is not doing well?

Brett: I would say, again, read the data. The Atlantic States Marine Fisheries manages the horseshoe crabs up and down the Atlantic East Coast, and individual states use those management directives to manage their own fisheries. States have the power to manipulate let's say, a quota. For instance, Massachusetts has a quota of 330,000 crabs that are allowed for bait. Massachusetts Division of Marine Fisheries cuts that down to about 165,000. I would let people know that there is a lot of people, a lot of scientists, a lot of biologists looking at the horseshoe crab fisheries on both the coast-wide level and the state level, and the populations are healthy.

In the Delaware Bay region alone, it's estimated that there's 30 million to 40 million adult crabs, countless juveniles, and it's probably safe to say that there's hundreds of millions of horseshoe crabs up and down the East Coast. The population itself is healthy. That said, horseshoe crabs are slow to mature. They are sexually mature at 10 to 12 years of age, so they're an animal that deserves and should be regarded as a fishery that needs to be managed. We need to monitor them, we need to look at them, and we want to minimize the impact that we do have.

Certainly, there are areas of concern. There are areas where population trends are not going the way we would like them to, and management addresses those through the process of fisheries management, again, on both the state and coast-wide level. I would encourage people to look at the data available with their individual states with the ASMFC and see the studies that are being done and see that there's a very healthy population out there.

What sort of conservation measures are being taken to help the horseshoe crabs?

Brett: Along those lines, again, the ASMFC regulates it. One of the things that they did in 2011 was to look at industry standards with the biomedical companies, and they put out a series of guidelines for the biomedical companies. They put in measures to reduce the bait harvest to a sustainable level. The goal of ASMFC is sustainable fisheries. Individual states can enforce two measures such as lunar closures like in Massachusetts and Rhode Island, where they protect the crabs during their most vulnerable time when they're up on the beaches spawning. There's no harvest allowed.

States implement things like size limits, bait bags that reduce the number of horseshoe crabs that are used by conch fishermen. There are areas that are off-limits to fishing. There are sanctuaries. There are national parks. There are areas that are off-limits to fisheries. There are conservation efforts along coast-wide levels. There are conservation efforts in place by the state and the manufacturers themselves. We work with some of the bay fishermen. In many cases, they self-regulate and look at the size crabs they have.

Certainly, ACC has taken an active part in conservation. Twenty or 30 years before fisheries were really managed, we had size limits. We had a return-to-sea policy with horseshoe crabs. Then most recently in the past

four years, we've had an aquaculture project in place. It's our horseshoe crab sustainability project, where we're actually growing horseshoe crabs and releasing them to the wild.

Tell us more about that project. What have you been doing to ensure horseshoe crab sustainability?

Brett: This is a really neat project, and I'm happy to be part of it. We see a lot of horseshoe crabs come through our facility. In around 2017, we had the idea of, "What if we did some *in vitro* fertilization of eggs? We can harvest eggs from the crabs fairly naturally. We're not harming the crabs. We take a few grams out, we can stimulate, and get the gametes from males and females. We can fertilize them *in vitro*, and then we can hatch them out."

We've set up now a patent-pending system that we designed. The company was behind us financially, provided us with the resources that we needed, and we created this system that's very easy to increase and decrease the number of crabs and eggs that are available. Ultimately, what we do is feed the system eggs, hatch out the horseshoe crabs and grow them through their first and second instars till they're about the size of a pencil eraser, and then we can release them to the wild. We've got an aquaculture permit, a one of a kind from the DMF in Massachusetts. They give us three bodies of water to release. I'm happy to say that this year, we released our millionth crab to the wild, which is something that is worthy of celebration, I think.

Has COVID had an impact on the demand for LAL?

Brett: Another great question that we hear a lot, and honestly, no, it hasn't had a great impact. Certainly, COVID did impact the way we do business, a lot more stuff from home, and whatnot, but the demand for LAL does not increase exponentially. Despite the fact that the vaccines are in production, treatments for COVID are in production, one has to remember that the pharmaceutical industry has the ability to scale up significantly. Whether you're testing 1 liter, or 10,000 liters of water per se, the same amount of LAL can be used. That scalability is critical to the pharmaceutical industry being able to supply an increased number of vaccines, for instance, or IV solutions, for instance, but the demand for LAL can remain relatively the same.

If people want to know more about the different projects you talked more about and also about horseshoe crabs and about endotoxin in general, and the different testing processes out there. Where can they get more information?

Brett: Well, certainly, our website, www.acciusa.com, we've got a lot of information there about our sustainability practices, some of our products, including the new PyroSmart NextGen, which is our exciting recombinant product. Some of the data I talked about today is available with the Atlantic State's Marine Fisheries Commission, Massachusetts division of Marine fisheries, and there, you can look at population trends and learn a lot about horseshoe crabs.