

# The Expanding Role of LAL Reagent in the Global Pharmaceutical Industry

**Jim and Frances Cooper**  
**Greensboro, NC**

**Benjie Swan**  
**Cape May, NJ**

# Horse Crabs as a Research Model

- 350 Millions years old
- Found up and down the Atlantic Coast
  - Northern Maine to Mexico
- The Atlantic Coast horseshoe crab, *Limulus polyphemus*, is the single most-studied invertebrate animal in the world

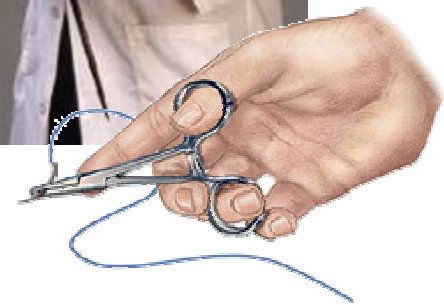
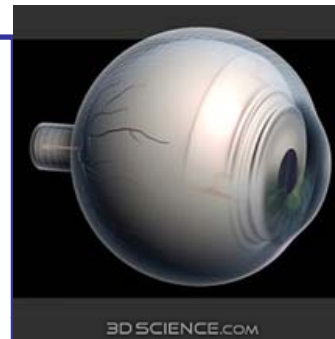


Oldest known Horseshow Crab



# Horseshoe Crabs as a Research Model

- Researchers have studied:
  - ecology and behavior
  - benefits to humans
- Thanks to the horseshoe crab, medical science has made great strides in:
  - eye research
  - development of surgical sutures and wound dressings
  - detection of bacterial contamination in drugs
- Three Nobel Prizes in Physiology or Medicine



# *Limulus amoebocyte lysate* (LAL)

The Limulus Amoebocyte Lysate test uses the blood of horseshoe crabs and is a major tool in the development of safe and effective injectable drugs and vaccines. LAL ensures the absence of potent fever producing environmental contaminants that are produced by Gram-Negative bacteria.



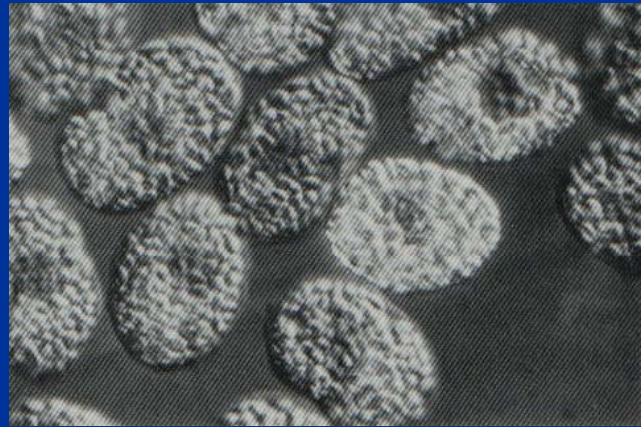
- In the early 1950's Dr. Fredrick Bang observed
  - clotting agent
  - primitive immune system.
  - Gram negative bacteria (GNB)
- In 1971, Cooper & Levin published on GNB detection with LAL test
  - Sensitive & specific for endotoxin
  - Injectable drugs and biologics release test



- Drugs were tested on rabbits to see if they developed a fever.
- In 1987, the U.S. Food and Drug Administration (FDA) accepted the LAL as a standard test for endotoxins.
  - **Replacing** rabbit tests for many clinical and biomedical applications.



# Limulus Amebocyte Cells

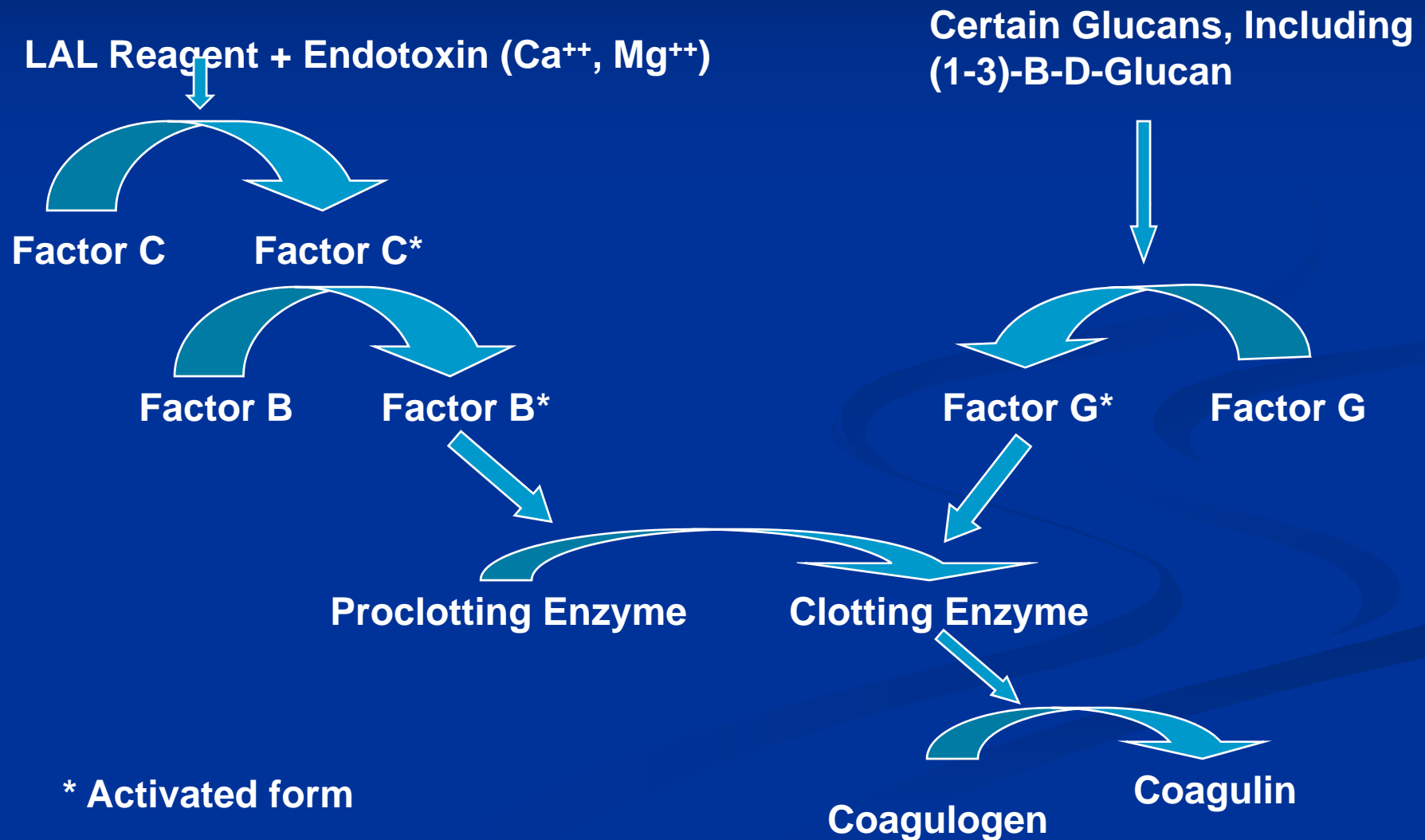


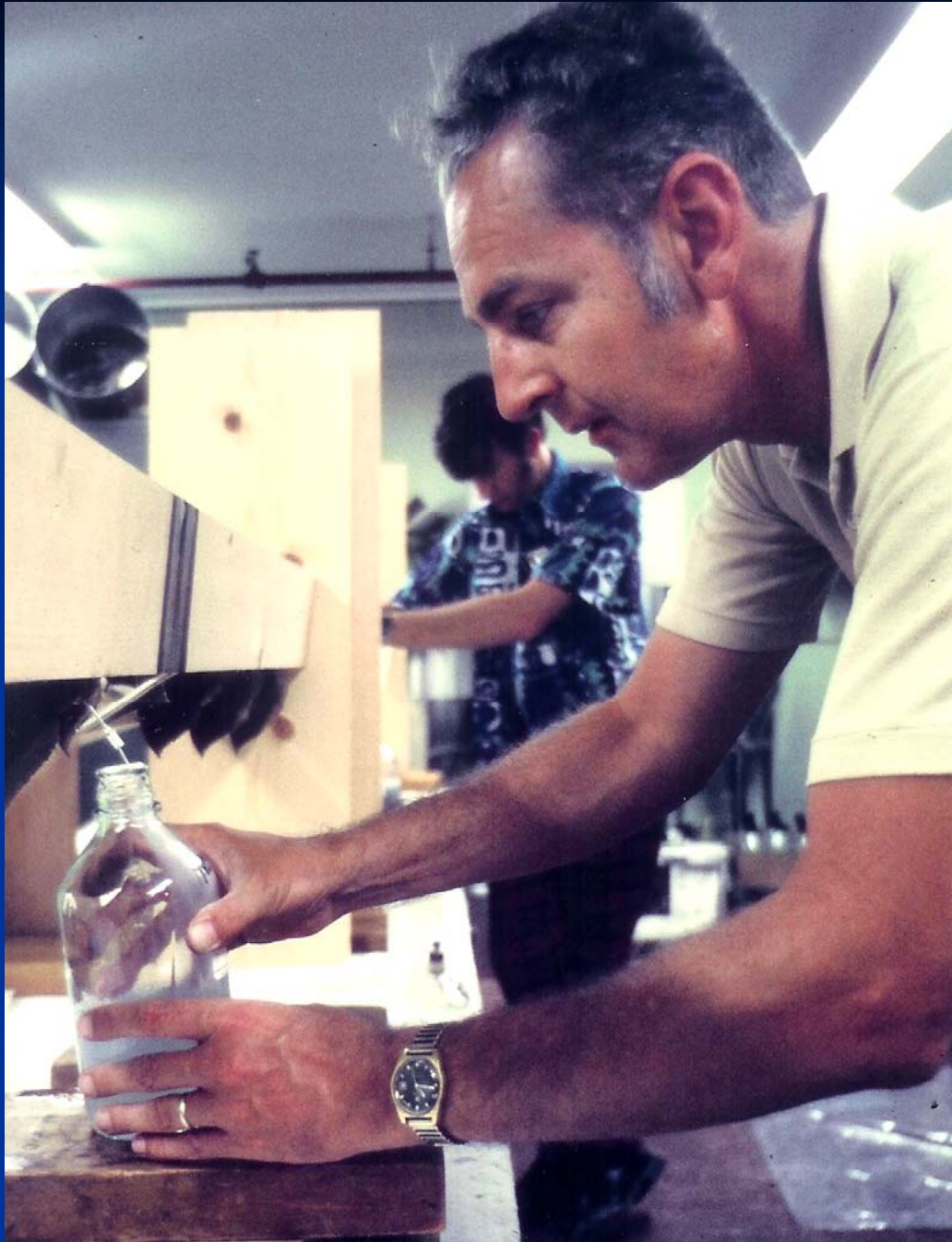
From: Peter B. Armstrong © 1979

Alan R. Liss, Inc., 150 5<sup>th</sup> Ave.

New York, NY 10011

# LAL Enzymatic Clotting Cascade: Basis for the Endotoxin Test





## Wallops Island, VA June 1972

Jim Cooper-FDA

Don Hochstein (NIH-DOB)

Ron Elin (NIH-NIAID)

# EVOLUTION of LAL

Collecting blue blood  
Donation from  
*Limulus*  
(horseshoe crab)  
in cleanroom area





**The crab with the blue blood:  
copper transports oxygen**

# Harvesting the Horseshoe Crab

## Harvesting

- Endosafe harvests Horseshoe crabs by hand
- Once the crabs are caught, they are secured and transported to the laboratory from the fishing pier by truck.
- As long as the animals are kept cool and moist during transport, they exhibit no adverse affects.

## Why is this important?

- Crabs are inspected for signs of responsiveness, good health and injury because the blood from one unhealthy crab can contaminate valuable, pooled LAL reagent.
- Only healthy crabs are used as donors.



# Horseshoe Crab Handling



- Always be careful when washing the crabs.
  - Carefully handle the crab by supporting the helmet-like shell and the tail.
  - Never lift the horseshoe crab by its tail because of risk for injury.

Proper handling of the crabs is vitally important. While the mortality rate is very low (<10%), this is DIRECTLY related to handling, not the bleeding process.

# Bleeding



## Bleeding

- Horseshoe crabs are passively bled using a stainless steel needle that is inserted into their circulatory system.
- Blood volume rebounds very quickly; procedure is innocuous.

## Why is this important?

**DID YOU KNOW ...** The amoebocyte is the crab's **ONE** and **ONLY** blood cell.

- Horseshoe crabs are not harmed during this process, and studies have indicated that there is no difference in mortality rate between bled and unbled horseshoe crabs.



## **Limulus prepared for bleeding**



**After donating their blue blood, Limulus are returned to the sea.**

# Conservation Initiatives Used by LAL Manufacturers

- Beginning in the 1980s, studies of HSC migration patterns in Delaware Bay by Finn and Swan generated critical data for assessing HSC status
- Frances Cooper and leaders in SC Division of Natural Resources gained legislative approval in 1991 to regulate the HSC resource
- SC bill became a model for the current HSC Fisheries Management Plan
- Focus now is handler training to assure the same care is used in return as well as harvesting

# Growth of U.S. LAL Industry

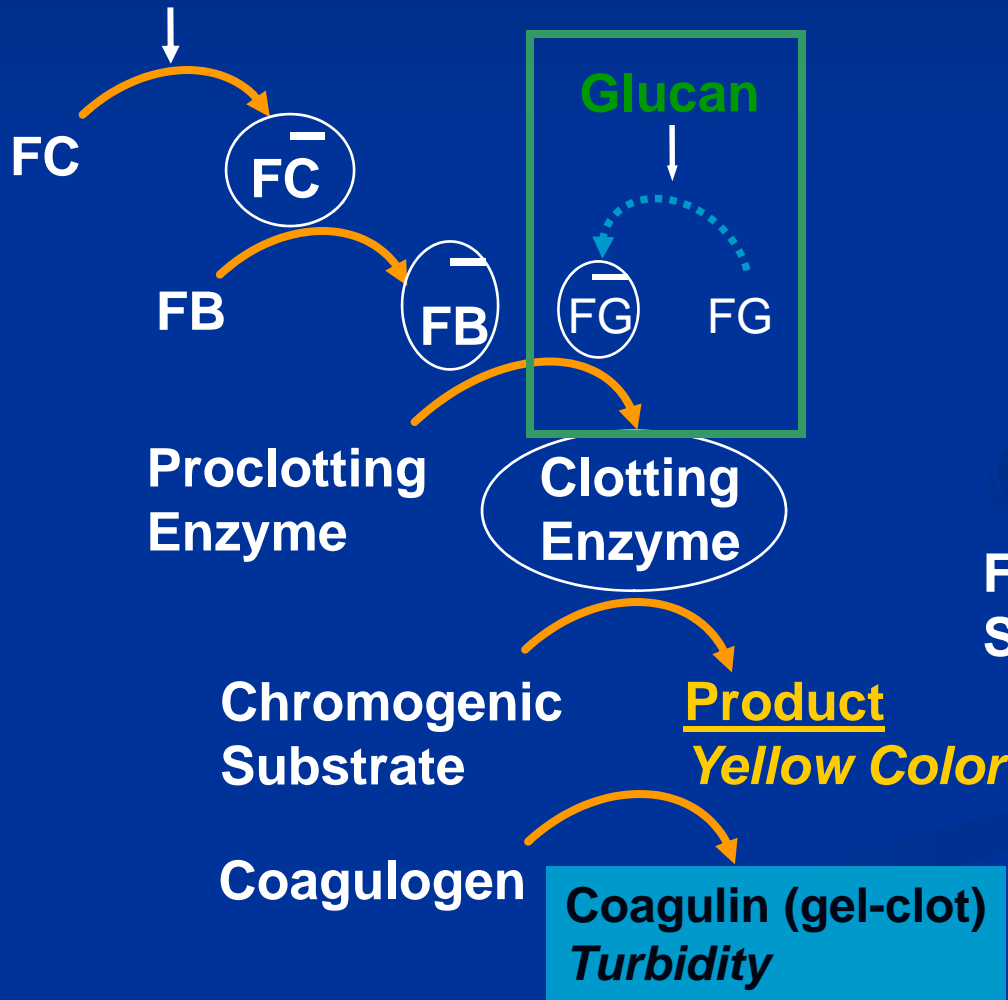
- Rapid growth after FDA approval in 1991 for LAL Reagent in US drug industry
- Endotoxin testing of injectables by LAL reagent required worldwide by 2002
- Global market now about \$100 million
- Asian production is negligible

# Current Need for Atlantic HSC

- At least 500,000 HSC will be needed annually to meet US demands for the foreseeable future
- HSC requirement are currently stabilized;
  - Use of more efficient, computer-assisted test methods in the pharmaceutical industry
  - Approval of cartridge LAL system
- Recombinant Factor C product has not impacted the LAL market

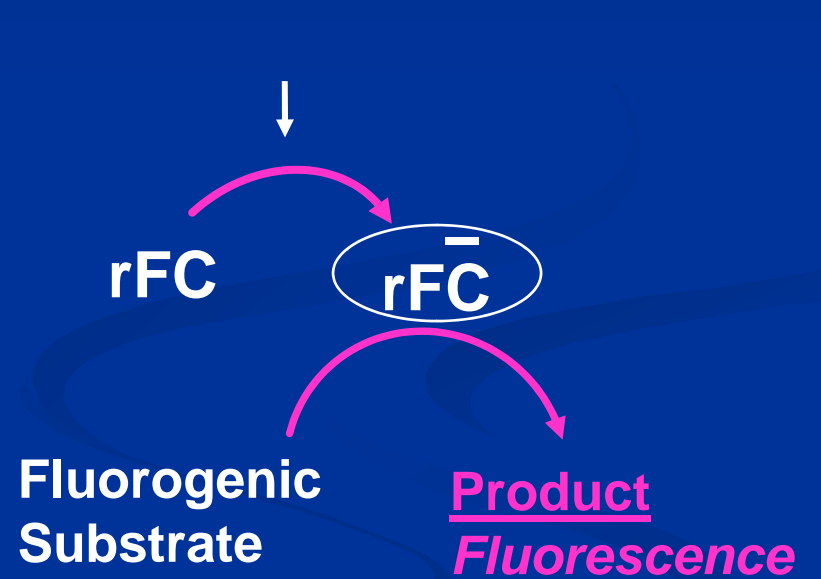
LAL  
Endosafe

Endotoxin

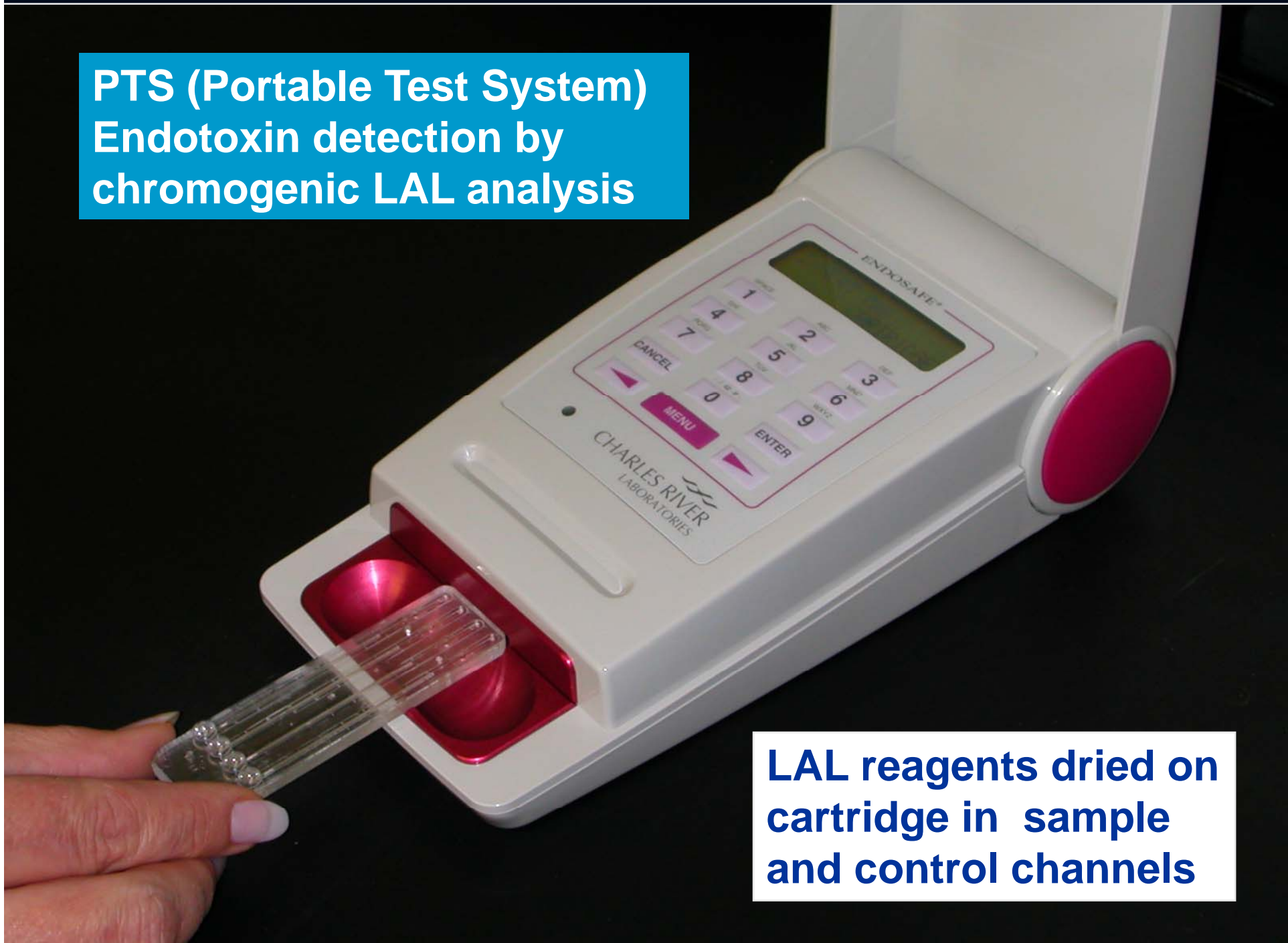


Recombinant Factor C  
Lonza

Endotoxin

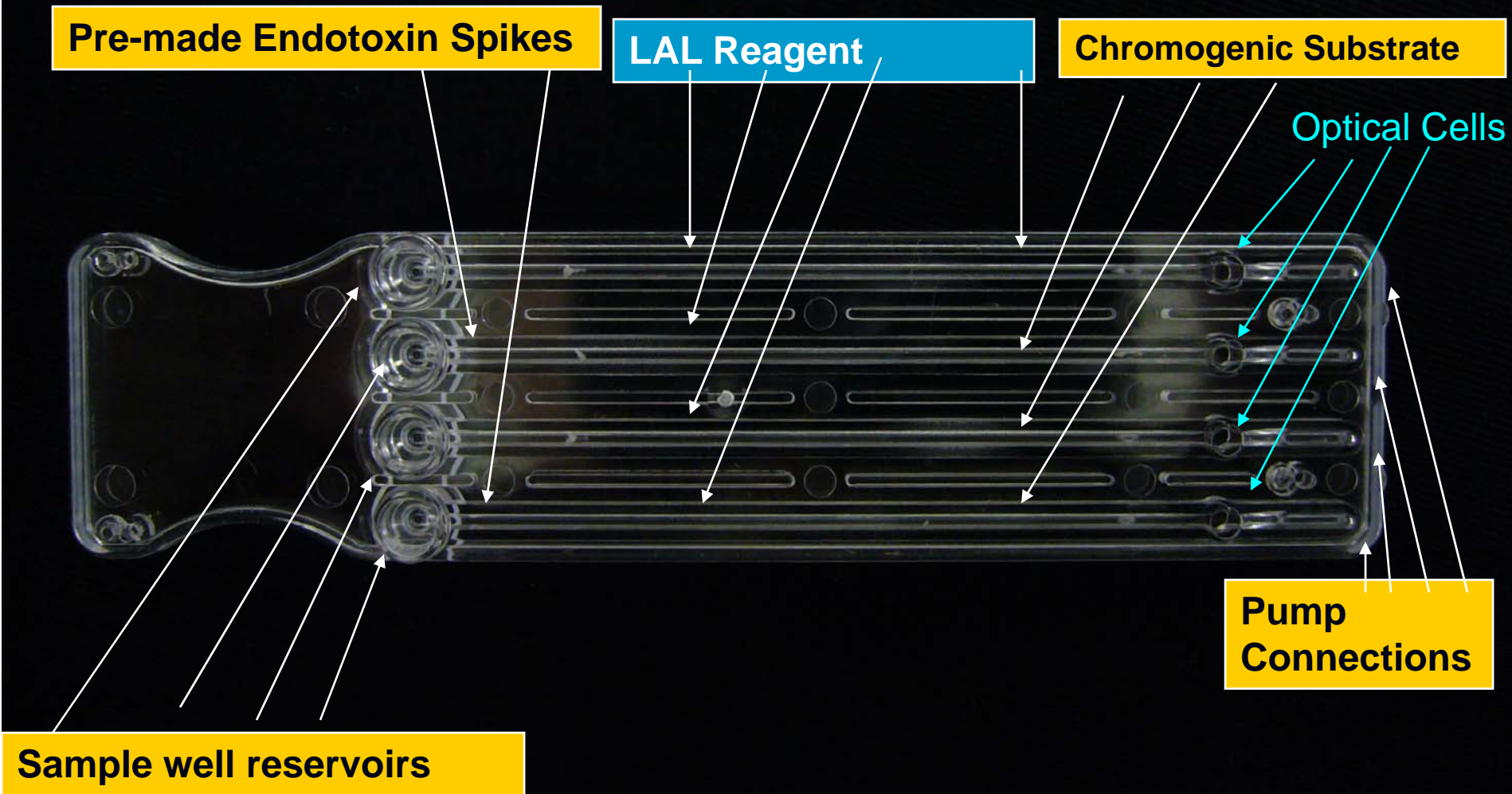


**PTS (Portable Test System)  
Endotoxin detection by  
chromogenic LAL analysis**



**LAL reagents dried on  
cartridge in sample  
and control channels**

# Cartridge Set Up – Duplicate Sample, Duplicate Spike; FDA-Approved Device



# Horseshoe Crabs as a Research Model

- In partnership with Charles River Endosafe, NASA's Marshall Space Flight Center in Huntsville, Alabama is developing a high-tech device which relies on four enzymes extracted from the blood cells of the horseshoe crab. It is very sensitive and can detect very low levels of bacteria within a sample and gives results in 5 to 15 minutes vs. two or three days in a traditional Petri dish culture. System monitors on-board water quality.



- In the future, new probes based on the horseshoe crab's blood may help space scientists search for primitive life on Mars and other planets.

# Summary

- Assuring the safety of injectable drugs by LAL is most important biomedical use of the Atlantic horseshoe crab
- Harvesting has stabilized at about 1/2 million HSC yearly due to more efficient use of LAL reagent and a new LAL cartridge system
- Focus on proper handling before and after processing limits mortality to 6-8% of harvested crabs