New test puts the squeeze on horseshoe crabs

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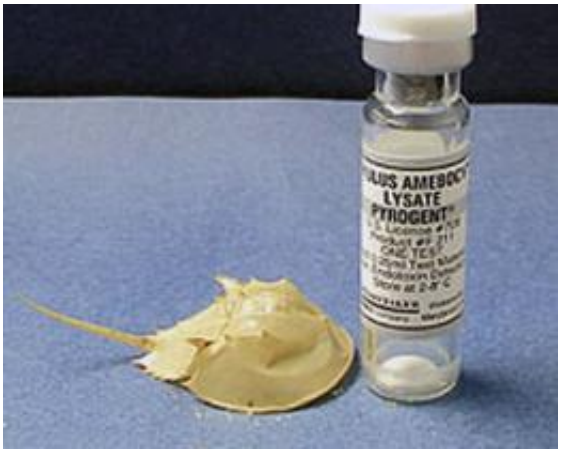

The shell of a juvenile horseshoe crab next to a bottle of limulus amoebocyte lysate, the gel obtained from horseshoe crab blood and used in medical testing. Credit: NOAA

If new technology under development proves out, horseshoe crabs will have to undergo fewer blood donations.

Horseshoe crabs routinely give up their blood to ensure human safety. Scientists use small amounts of the blood, which is colored blue, to test medicinal drugs and other items for the presence of endotoxins. These poisons associated with bacteria can harm human health.

A team at the University of Wisconsin-Madison, headed by engineering professor Nicholas Abbott, has discovered a new means of testing for endotoxins that involves inanimate compounds.

The Food and Drug Administration requires manufacturers to test every drug and medical device that it certifies for the presence of endotoxins. Called the limulus amoebocyte lysate assay, this "gold standard of testing" relies on exposing the drug under investigation to horseshoe crabs' blood. The blood clots when endotoxins are present.

"An enzyme of the horseshoe crab is activated in the presence of the endotoxin," explained Peter Armstrong, a biology professor and horseshoe crab expert at the University of California, Davis. "That activates another enzyme which activates a third which causes the blood clot."

The blood extraction process is relatively benign.

"We insert a large syringe into the crab's heart and take about one third or one quarter of the total blood out of the animal," Armstrong said. "The vast majority of the crabs survive it."

Despite the success of the lysate assay, scientific teams have explored alternative approaches to testing for endotoxins. Most of the methods to date have used biotechnology techniques to create substitutes for the horseshoe crabs' blood.

"But they haven't got very far," Armstrong said.

Abbott's method works differently. It relies on liquid crystals.

Familiar in flat-screen televisions and computer monitors, these forms of matter combine the mobility of typical liquids and the ordered structure of conventional solids. Changes in their structure alter their optical properties, giving them different appearances under a microscope.

"We've had an interest in the notion that one can use the properties

inherent in liquid crystals as amplifiers," Abbott said. "More recently we've had idea of using droplets of liquid crystals. When you confine a liquid crystal in a droplet you predispose it to responding to external stimuli."

Abbott thought that the geometry of droplets might give them a high sensitivity to lipids, the key components of endotoxins. Initial research showed that the endotoxins' lipids did indeed alter the appearance of liquid crystal droplets under a microscope.

"The organization of the liquid crystal inside the droplet changes. That changes the way the droplet scatters light," Abbott said."

In addition, Abbott's team reported in Science that "[W]e were surprised to find that we could decrease the concentration of the endotoxin to extremely low levels and still see that change in the ordering of the liquid crystals."

But what causes the change?

The team first thought that the surfaces of the droplets adsorbed the endotoxins, but the process was too sensitive to justify that explanation. So Abbott and graduate students I-Hsin Lin and Dan Miller determined that the endotoxins located themselves on minuscule structural defects that exist naturally in liquid crystal droplets.

However it works, the liquid crystal approach has a specific advantage over limulus amoebocyte lysate and similar tests: It does not require living organisms.

"The (limulus amoebocyte lysate) test is very useful and FDA-approved, but it is a biologically derived assay, so you have all the complexities associated with seasonal variations and other factors," Abbott said. "Our

approach is a demonstration of principle. But it doesn't require any biological reagent, it doesn't need to be refrigerated, and it could be fairly cheap and robust."

Abbott emphasized that his team's technology remains in a preliminary stage.

"We have found a fundamental phenomenon," he said. "But it's a long path to have a validated technology that can replace the horseshoe crab assay."

So for now, horseshoe crabs will continue to donate their blood to the cause of human safety.

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