In the summer of 2006, students at the University of Toledo’s Lake Erie Center made a ghastly discovery on the beach across the street: dead fish washed up on the shore, and struggling fish in the shallow water, all showing bright red hemorrhages on their sides. The students and their instructor, Dr. Carol Stepien, later found out that they had witnessed the first outbreak of viral hemorrhagic septicemia virus, or VHSV, in western Lake Erie.
“VHSv appears kind of like ebola for fish, where they get lesions and bleed out,” says Stepien, Director of the Lake Erie Center and Professor of Ecology at the University of Toledo (UT). “It also was very virulent, like ebola. A lot of dead fish, including yellow perch and drum, were washing up on the beaches during these scattered outbreaks.”

Of course, anything that could impact Lake Erie’s fishing industry needs to be addressed. Almost seven years after that initial encounter, Stepien and her research partner Dr. James Willey from the UT Health Sciences campus, her PhD student Lindsey Pierce, and their technicians Erin Crawford and Vrushalee Palsule have developed a new rapid test for VHSv that is not only much faster than the traditional cell culture method, but also has a much higher degree of accuracy. The test is based on a procedure called StaRT-PCR (standardized reverse transcriptase polymerase chain reaction, a DNA-based testing method) developed by Dr. Willey, that is very accurate, since it uses built-in internal controls. The research group has now adapted their test to use a qRT-PCR (quantitative real-time polymerase chain reaction, another DNA-based method) platform, as that equipment is already a part of most fish diagnostic labs.

Other assays that have been developed for VHS include qRT-PCR tests that have a false negative rate of 14-47%, since they lack built-in internal controls. And cell culture assays, which are the standard method of virus testing, can take a month or more to run, making results untimely at best, and can have false negative rates of as high as 76%, according to previous research. “It’s a long procedure, and what we were told by agencies and other researchers was to try to develop an accurate, low-cost test,” Stepien relates.

And that’s what the team did, with funding from Ohio Sea Grant and the USDA. “We’ve developed a very rapid and accurate test that can tell every strain of VHSv from all over the world,” explains Stepien. “We can diagnose VHS 100%, and our test is negative for any other type of fish virus or human virus, so it’s very accurate.”

The test takes only a few hours, instead of a month or more with cell culture, and includes a built-in control that compares the study sample with a standardized virus sample. This ensures that a positive result is a true positive, and not the false negative often seen without an internal control. The test also allows researchers to determine the quantity of virus present in the sample, which can be an indicator of whether an infection is latent – not causing any symptoms – or if it’s an acute infection that will soon kill affected fish.

In addition to developing a hands-on testing method for VHSv, Stepien and graduate student Lindsey Pierce have also traced the evolution of VHSv strains across the globe since its discovery in marine organisms. “VHSv was known since the 1920s in Europe,” Stepien explains. “It was found in rainbow and brown trout in aquaculture, where you’re keeping fish in very close captive environments, and they can easily transmit a virus from one to another.” Here, the effects of VHSv can be especially devastating, and efficient detection of the virus and determination of which fish are infected is essential to prevent economic losses.

VHSv in the wild is spread especially during spawning season in the spring and early summer, when fish congregate in large numbers and remain in close contact for a few days. The virus is transmitted via mucus and other secretions, and can survive in cool water for up to 11 days, which allows it to be transported from one region to another via water currents.

Bait fish have also historically been shown to transmit VHSv from one body of water to another, leading to an agreement between Great Lakes states and Canadian provinces – part of the 2011 Aquatic Invasive Species Action Plan – to require fish to be certified as VHSv-free before they can be transported. Currently, a month-long cell culture is recommended for this certification, which significantly delays shipments.

Stepien is now collaborating with a number of other researchers on large-scale evaluations of the testing procedure to determine whether it could eventually replace the cell culture standard. Dr. Yan Zhang at the Ohio Department of Agriculture and Dr. Mohamed Faisal at Michigan State University, who performs testing for the state of Michigan and the upper Great Lakes, will both implement the new method in their labs to determine how well the test fares in a non-experimental setting.

Over recent years, an increasing number of fish species have tested positive for VHSv, but haven’t shown symptoms, which indicates that fish may have developed resistance to the virus. However, these host fish can still spread the virus to other fish that may be more susceptible to infection, making accurate diagnosis and efficient preventive testing a priority for natural resource managers and fish farmers. And that’s exactly what Stepien thinks this test will be able to do.

“We can test for VHSv with very high accuracy, and we’re hoping that it’s going to be very low cost, and easy to do,” Stepien says. “It’s been very fun to figure out the evolution of this virus. We’ve discovered all kinds of cool things about it.”

For more about this Ohio Sea Grant-funded project, contact Dr. Stepien at carol.stepien@utoledo.edu.