Quick test finds signs of diarrheal disease

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Strips developed at Rice University to look for the diarrheal disease cryptosporidiosis show the difference between positive and negative test results. Control stripes on the right show both tests are valid, while the presence of a second stripe near the center of the top strip shows that parasites are present in a patient. Credit: Zachary Crannell/Rice University

Bioengineers at Rice University and the University of Texas Medical Branch (UTMB) at Galveston have developed a simple, highly sensitive and efficient test for the diarrheal disease cryptosporidiosis that could have great impact on global health.

Results from the diagnostic developed by the lab of Rice bioengineer Rebecca Richards-Kortum are read from a paper strip that resembles a pregnancy test. Lines on the strip tell whether samples taken from the stool of a patient contain genetic DNA from the parasite that causes the disease.

The research is detailed online in a new paper in the American Chemical Society journal *Analytical Chemistry*.

"Diarrheal illness is a leading cause of global mortality and morbidity," said Richards-Kortum, director of the Rice 360°: Institute for Global Health Technologies. "Parasites such as cryptosporidium are more common causes of prolonged diarrhea. Current laboratory tests are not sensitive, are time-consuming and require days before results are available. A rapid, affordable, accurate point-of-care test could greatly enhance care for the underserved populations who are most affected by parasites that cause diarrheal illness."

A. Clinton White, director of the Infectious Disease Division at UTMB, asked Richards-Kortum to help develop a diagnostic test for the parasite. "I've been working with cryptosporidium for more than 20 years, so I wanted to combine her expertise in diagnosis with our clinical interest," he said. "Recent studies in Africa and South Asia by people using sophisticated techniques show this organism is a very common, underappreciated cause of diarrheal disease in underresourced countries."

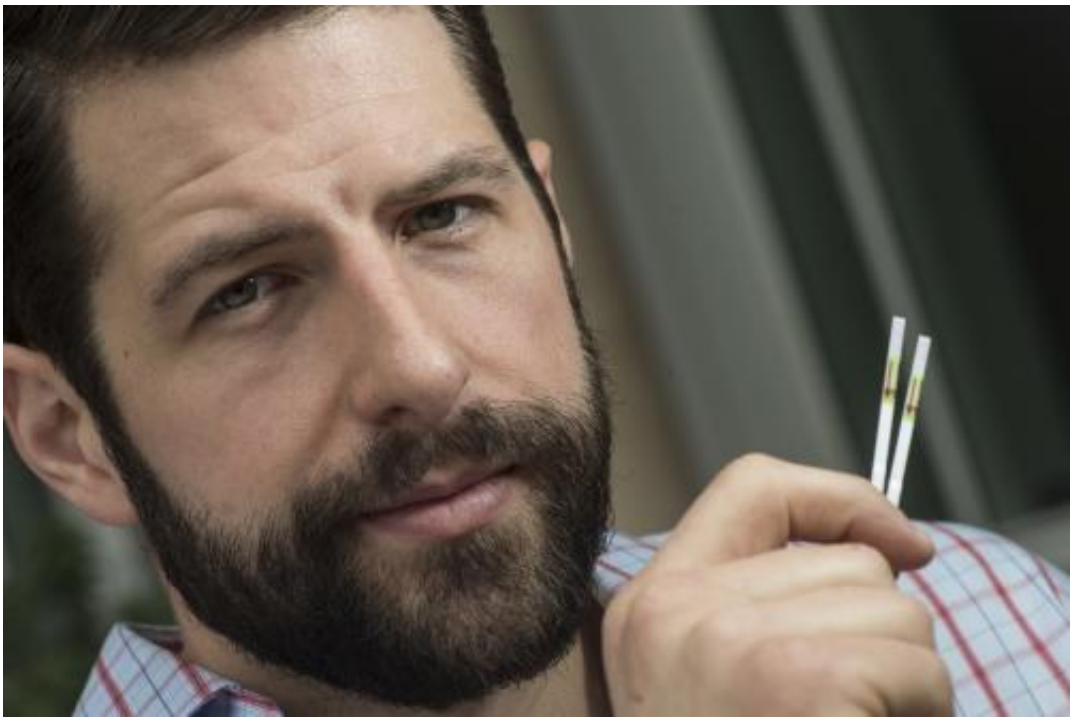
The parasite is common in the United States, he said, but less than 5 percent of an estimated 750,000 cases are diagnosed every year. In 1993, an outbreak of cryptosporidium in the water supply sickened 400,000 people in Greater Milwaukee, he said.

Lead author Zachary Crannell, a graduate student based at Rice's BioScience Research Collaborative, said the disease, usually transmitted through drinking water, accounts for 20 percent of childhood diarrheal deaths in developing countries. Cryptosporidiosis is also a threat to people with HIV whose immune system is less able to fight it off, he said.

"In the most recent global burden-of-disease study, diarrheal disease accounts for the loss of more disability-adjusted life years than any other infectious disease, and cryptosporidiosis is the second leading cause of

diarrheal illness." Crannell said. "Anybody, if it's not treated, can get dehydrated to the point of death.

"There's a lot of new evidence that even with asymptomatic cases or cases for which the symptoms have been resolved, there are long-term growth deficits," he said.



Rice University graduate student Zachary Crannell displays strips used to diagnose cryptosporidiosis, a parasite that causes diarrheal disease. The test developed by Rice and the University of Texas Medical Branch relies on the amplification and detection of DNA from parasites that cause the disease. Credit: Jeff Fitlow/Rice University

Current specialized tests that depend on microscopic or fluorescent analysis of stool samples or polymerase chain reactions (PCR) that amplify pathogen DNA are considered impractical for deployment in

developing countries because of the need for expensive equipment and/or the electricity to operate it.

The Rice test depends on recent developments in a recombinase polymerase amplification (RPA) technique that gives similar "gold standard" results to PCR but operates between room and body temperatures. In Rice's experiments, samples were prepared with a commercial chemical kit that releases all the DNA and RNA in the small amount of stool tested. The purified nucleic acids are then combined with RPA primers and enzymes tuned to amplify the pathogen of interest, Crannell said.

"If the pathogen DNA is present, these primers will amplify it billions of times to a level that we can easily detect," he said. The sample is then flowed over the detection strip, which provides a positive or negative result.

The RPA enzymes are stable in their dried form and can be safely stored at the point of care without refrigeration for up to a year, he said.

While current tests might catch the disease in samples with thousands of the pathogens, the Rice technique detects the presence of very few – even one – parasite in a sample. In their experiments, the researchers reported the presence or absence of the disease was correctly identified in 27 of 28 infected and control-group mice and all 21 humans whose stool was tested.

Crannell said the method requires little equipment, because the enzymes that amplify DNA work best at or near body temperature. "You don't need a thermal cycler (used for PCR analysis); you don't need external heating equipment. You can hold the sample under your armpit, or put it in your pocket," he said.

The research team's goal is to produce a low-cost diagnostic that may also test for the presence of several other parasites, including giardia, the cause of another intestinal disease. The researchers are working to package the components for use in low-resource settings, Crannell said.

Co-authors are Rice graduate student Brittany Rohrman, and research scientist Alejandro Castellanos-Gonzalez and lab technician Ayesha Irani of UTMB. White is a professor and chief of the Department of Internal Medicine at UTMB. Richards-Kortum is Rice's Stanley C. Moore Professor and chair of the Department of Bioengineering and director of Beyond Traditional Borders as well as Rice 360°.

More information: Paper: pubs.acs.org/doi/abs/10.1021/ac403750z

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