Rapid Detection of Waterborne Pathogens in Drinking Water

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Environmental Problem

Drinking water in the United States is among the safest in the world. Despite that, undetected microbial contamination can lead to serious illness and death. One such pathogen, *Cryptosporidium parvum*, can cause debilitating diarrhea leading to serious illness. A number of outbreaks of cryptosporidiosis have been reported in the United States, including a major outbreak in Milwaukee in 1993 that killed more than 100 people. To monitor drinking water for the presence of *C. parvum* oocysts, water utilities often rely on collecting water samples that then are submitted to an outside reference laboratory for testing. Besides the expense of $350–$650 per analysis, public health is jeopardized by a delay of up to 10 days in obtaining the final results.

Another problem inherent in current testing methods is that determination of the viability status of the oocysts requires additional complicated tests. As effective methods to inactivate *C. parvum* are implemented on a widespread basis in water utilities, it will be even more important to determine the viability of microbes that manage to enter the plant's distribution system, whether in an active or inactive state.

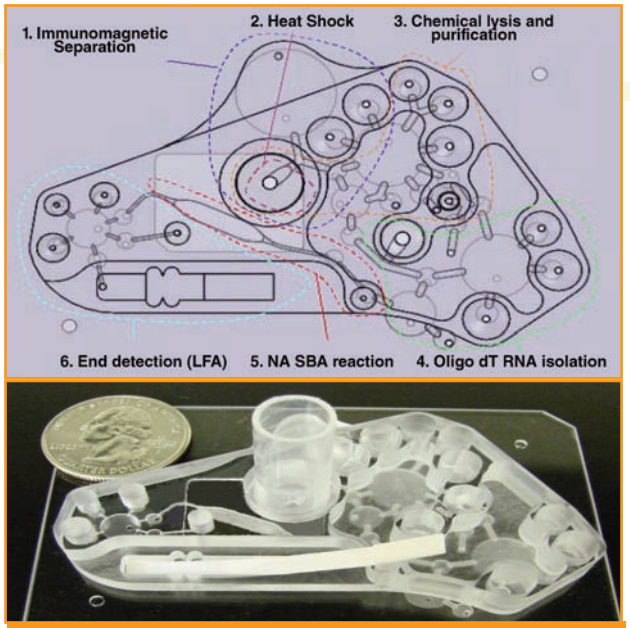
SBIR Technology Solution

With support from EPA's SBIR Program, Rheonix has developed a fully automated and rapid molecular diagnostic system that is able to detect single oocysts of *C. parvum* in drinking water and distinguish viable from nonviable oocysts. Moreover, its patented Chemistry And Reagent Device (CARD™) is able to automatically perform all sample preparation, analysis, and readout without user intervention. A bench-top assay was originally developed by Innovative Biotechnologies International, Inc. (IBI), prior to its acquisition by Rheonix in 2008, that could be completed within 4-6 hours. Considerable direct intervention, however, was required. Those steps included: (1) immunomagnetic separation and washing of oocysts; (2) heat-shock induction of the *hsp*70 mRNA response to differentiate viable from nonviable oocysts; (3) lysis and purification of oocysts; (4) extraction and purification of mRNA; (5) nucleic acid sequence-based amplification (NASBA) gene amplification of the target gene sequences; and (6) detection of the NASBA amplicons on a lateral flow system utilizing liposomes, conjugated to molecular probes, that also encapsulate signal-generating molecules to provide an inexpensive method to detect the amplicons.

The bench-top assay was adapted to Rheonix's fully integrated CARD™ platform, which analyzes clinical specimens automatically. Once a "raw" water sample is applied to the *Crypto*Detect CARD™, required steps are performed seamlessly and automatically. The ease-of-performance reduces the currently high costs associated with monitoring drinking water for the presence of *C. parvum* and significantly reduces the level of training required, providing time and cost benefits in water treatment plants' testing of drinking water for microbial safety.

Commercialization Information

As a result of EPA's SBIR funding, Rheonix currently is preparing *Crypto*Detect CARD™ devices and the software-interfaced control system for evaluation by Battelle Memorial Institute as part of EPA's Environmental Technology Verification (ETV) testing program.

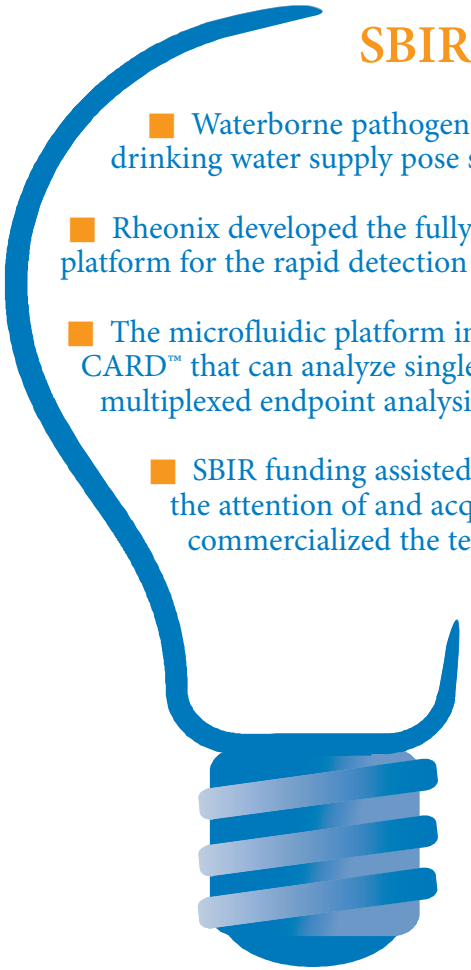


Top: **Schematic of Card™ showing 6 pumps and 25 valves and general locations of the various functional components of the assay.** ***Bottom:*** **Photograph of fully integrated *Crypto*Detect CARD™.**

Simultaneously, Rheonix is pursuing strategic relationships with companies that actively sell to and service the drinking water industry. The ideal partner for Rheonix is a company that not only maintains a dominant presence in the marketplace but also has complementary products whose sales can be leveraged by the availability of the unique *Crypto*Detect CARD™. The Company will continue to collaborate with EPA to achieve the necessary regulatory approvals to permit the *Crypto*Detect CARD™ to be implemented on a nationwide basis, thereby further improving the safety of the U.S. drinking water supply.

Company History

Rheonix began its microfluidic efforts approximately 6 years ago as the microfluidic division of KIONIX, Inc. (Ithaca, NY), a global leader in the design and fabrication of high-performance, silicon-micromachined Micro-Electro-Mechanical Systems (MEMS) inertial sensors. After spinning out of KIONIX and acquiring IBI in December 2008, Rheonix has continued its development of the fully integrated CARD™ platform for molecular diagnostics. Numerous CARD™ devices have been developed for use in both human *in vitro* diagnostics markets, as well as the drinking and recreational water testing markets. An existing bench-top assay generally can be migrated and prototyped on a CARD™ device within 6-12 weeks. Having recently raised equity capital, the company has adequate financial resources to complete the necessary development and regulatory approval processes to permit it to offer revolutionary products in multiple market niches. In addition to EPA funding, the company also is the recipient of grants from the National Institutes of Health (NIH), National Science Foundation (NSF), and New York State Energy Research and Development Authority (NYSERDA).



SBIR Impact

- Waterborne pathogens, such as *C. parvum*, that enter the U.S. drinking water supply pose significant hazards to public health.
- Rheonix developed the fully automated and customizable CARD™ platform for the rapid detection of *C. parvum* oocysts.
- The microfluidic platform incorporates a patented, low-cost, disposable CARD™ that can analyze single or multiple raw clinical samples and provide multiplexed endpoint analysis.
- SBIR funding assisted IBI to develop its bench-top assay and attract the attention of and acquisition by Rheonix, which improved and commercialized the technology with additional EPA SBIR support.