A Hybrid Pathogen Detection System For Drinking Water

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Environmental Problem

Cryptosporidium parvum oocyst is a resilient waterborne protozoan that resists ordinary water purification treatments. It causes a gastrointestinal disease called cryptosporidiosis which is one of five major causes for 5 million deaths each year worldwide. *Cryptosporidium parvum* oocysts are of particular interest in the water industry because the infectious dose is low (i.e., 1 to 132 ooycsts) and it does not respond to common drug treatments. Recently, a *Cryptosporidium* outbreak sickened more than 1,700 people, mostly children and teenagers, in New York.

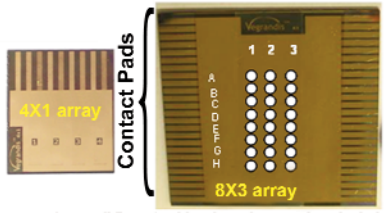
Under new requirements of the Drinking Water Act, water systems using sand or diatomaceous earth filtration, those required to filter and use conventional/direct filtration, and those using alternative filtration must install and operate a technology that reliably achieves 99 percent removal of *Cryptosporidium* oocysts. To achieve these results, a reliable technology with very low detection limits that eliminates false positives and false negatives must be developed and made readily available to measure the levels of *Cryptosporidium oocysts* in public water systems, including surface water supplies, water treatment plant process streams, and distribution systems. Vegrandis' technology is appropriate for these needs.

SBIR Technology Solution

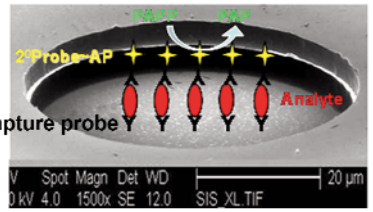
Assays for waterborne pathogens using microelectrochemical detection are desirable because the microelectrochemical signal is dependent only on the loss or gain of electrons from the electroactive enzymatic product and is not affected by the sample color or turbidity minimizing pre-treatment procedures that are usually performed to eliminate false positives and false negatives. Self-contained microelectrochemical array assay platform technology developed by the company's Chief Technology Officer and her collaborators at the University of Arkansas has been licensed exclusively to Vegrandis. This technology offers advantages over existing methods and is expected to provide the following key benefits:

- Rapid assay at < 30 min with a detectable signal 30 seconds after incubation with the precursor to the reporter molecule.
- Improved sensitivity that arises from the use of microelectrodes which generally show a high signal-to-background ratio.
- Low minimum detection concentration (i.e., 9 pg/mL or 56 x 10^{-15} moles/L IgG).
- Improved reliability of results (standard error of 1 to 3%) that arises from the reproducible covalent immobilization of the capture of the monoclonal IgG antibody on the solid platform.
- Large range of assay volumes (from 100 nL to > 35 mL).
- Flow-through device capable of capturing pathogens from large sample volumes (i.e., up to 10 L).
- Use of inexpensive portable, lightweight, permanent equipment (< 3 lbs; > $3,000 to < $10,000) with disposable assay cartridges.
- Multiple pathogens can be assayed simultaneously in the same analysis event on the same sample and can be detected simultaneously.
- Automated for high throughput analysis of multiple samples and ease of use by nonskilled personnel.
- Elimination of false positives and false negatives.
- Unaffected by color and turbidity.
- Ability to assess precision and accuracy with quality control and proficiency test samples.

A Chips with arrays of microwells (schematic shown on 8x3) that have three individually addressable electrodes



B Microwell ELISA with microelectrochemical signal detection



Bottom electrode for capture, middle electrode for detection, top electrode as reference/counter electrode

Figure A. The two microcavity array chips.

Figure B. Scanning electron micrograph of a 50-micron diameter cavity on a chip with a schematic of the sandwich type enzyme linked microelectrochemcial immunoassay detection of *Cryptosporidium parvum* oocysts.

To use the Vegrandis Automated Electrochemical Analyzer, the user places the sample in the sample reservoir and the reagents in the reagent module, inserts the disposable cartridge in the analyzer, chooses the program, and clicks the start button. The instrument has built in programs for the flow injection system and for the electrochemical detection that will follow the protocol for the chosen assay. In less than 30 minutes, the LCD display will show the quantity of oocysts in the sample.

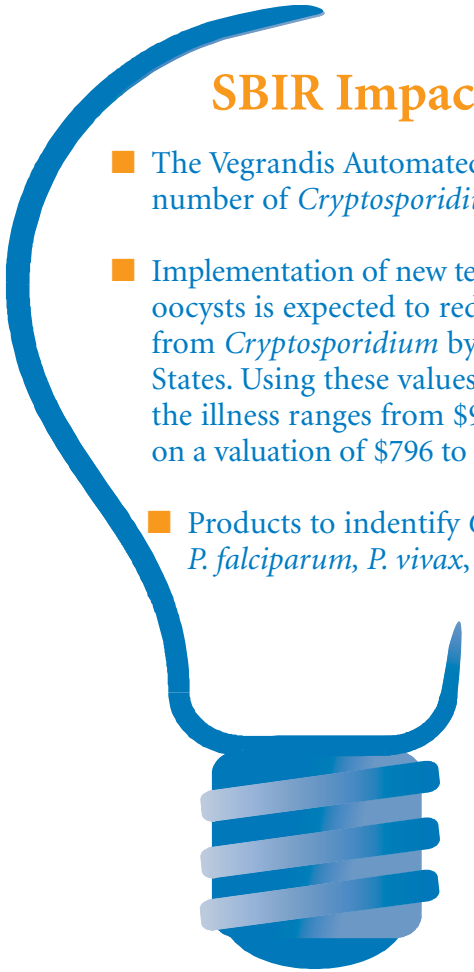
Commercialization Information

In 2007, Vegrandis released the first few products geared toward research application of the self-contained microcavity electrodes. Clients have included universities and small businesses developing microelectrochemical detection of various analytes. The next wave of products in development will be for environmental and water monitoring. The products will include the fully automated 24-channel electrochemical and 5-fluid delivery analyzer, and disposable cassettes for the detection of *C. parvum* and *G. lamblia*. These will be released toward the third quarter of 2008.

Other products in development are geared toward homeland security and clinical diagnosis uses. These products include a handheld 24-channel electrochemical analyzer, reagent kits, and disposable cassettes for the detection of *B. anthracis*, *B. globigii*, *ricin*, *E. coli, P. falciparum, P. vivax*, and ovarian cancer biomarkers.

Company History

Vegrandis was started in April 19, 2002 in Fayetteville, Arkansas. It was a spin-off from the University of Arkansas where the self-contained microelectrochemical devices were first developed by one of the company's founders, Dr. Ingrid Fritsch. The company's mission is to develop, build, and commercialize inexpensive, easy-to-use, sensitive, and portable instruments, microcavity arrays, laboratories-on-a-chip, and reagent kits for medical, environmental, and homeland security applications. The approach to achieve this mission is to use miniaturized devices that are reusable or disposable and are used with inexpensive easy-to-use automated instrumentation that requires only nanoliter volumes of reagents and samples. To date, the company has received numerous grants to support its work.



SBIR Impact

- The Vegrandis Automated Electrochemical Analyzer can quickly identify the number of *Cryptosporidium* oocysts in a water sample.
- Implementation of new technologies to remove 99 percent of *Cryptosporidium* oocysts is expected to reduce the mean annual number of endemic illnesses from *Cryptosporidium* by 12,000 to 41,000 cases per year in the United States. Using these values, the mean estimated annual benefits of reducing the illness ranges from $9.5 million to $58.3 million per year which is based on a valuation of $796 to $1,411 per incidence of cryptosporidiosis prevented.
- Products to indentify *G. lamblia, B. anthracis, B. globigii, ricin, E. coli, P. falciparum, P. vivax*, and ovarian cancer biomarkers are in development.