International Innovation

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A fully integrated assay and platform for detecting Clostridium difficile

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Easy on the stomach

Bruce Irvine explains the technology behind his PureLyse® method for cell lysis and DNA extraction and evaluates the challenge of creating an integrated assay that maximises on time, sensitivity and cost

Firstly, can you outline the primary goals of your research, and explain what inspired the creation of this initiative?

This project was inspired by the results that we were getting from preliminary data from testing our PureLyse® method of cell lysis and DNA extraction. The goals of this project are to:

- Develop a rapid DNA extraction method for *Clostridium difficile* (C. diff) from stool and swabs to be complete within five minutes and deliver DNA that is ready for amplification
- Develop an isothermal amplification of the Tcd B gene to detect down to 100 copies per 200 ul of sample. TcdA will also be detected as backup
- Identify the hypervirulent strain NAP1/027 by resolving TcdC del 117 Mut from Tcd C wildtype
- Integrate the functions of sample prep and amplification into a prototype disposable cartridge. Complete processing of sample preparation amplification and detection will be conducted within 15 to 30 minutes
- The Phase II goal is to develop the instrument that will automate the sample processing, amplification, and detection
- The long-term goal is to develop a point-of-care testing (POCT) system for detection of the high priority pathogens of enteric disease from stool samples

Could you offer an insight into the main obstacles in the current methods for detecting C. diff? Are these techniques time- and cost-efficient?

There are three PCR based assays on the market. The Becton Dickinson GeneOhm C. diff assay leverages real-time PCR to rapidly detect the toxin B gene in C. diff. In 2008, the cost per test was US $27 and runtime was 75-90 minutes. Disadvantages of the assay are the low ease of use from the additional required sample preparation steps and long runtime. The Cepheid Xpert C. diff assay utilises real-time optical detection and rapid thermocycling in one instrument for detection of the toxin B gene. The cost per test is higher than that of other competitors at $37.50, but it has the shortest runtime of the competition at less than 45 minutes. The Progastro Cd Assay requires real-time PCR to detect the toxin B gene in C. diff. Its cost per test is $25 and its runtime is three hours.

How will the integrated assay and platform succeed where current techniques have failed?

Sample prep has been rate limiting for getting sample to answer nucleic acid systems into the point-of care arena. Our simple PureLyse system will lower the obstacles substantially. PCR still requires very expensive instrumentation so going with an isothermal assay that is rapid lowers the cost and assay duration. Good candidate assays include NEAR, EXPAR, CPA, and LAMP.

How do you propose to distinguish between the hyper-virulent strain of C. diff and the wildtype? How will this help to mitigate further transmission and improve treatment for those infected?

There is a single base deletion in the TcdC gene that is a marker for this strain. In order to reduce selective pressure for vancomycin resistance in enterococci, current guidelines recommend the first-line use of metronidazole over vancomycin. However, recent reports suggest that the new hypervirulent strain may not respond as well to treatment with metronidazole15 despite the absence of laboratory evidence of metronidazole resistance16. This may be due to increased virulence in the new strain.

Indeed, metronidazole must be absorbed into the blood stream to be converted to its active form so patients with intestinal bleeding may not absorb the prodrug as

Pinpointing immediate results

A team of researchers at **Claremont BioSolutions**, California, is on the verge of introducing the first point-of-care system for detecting *Clostridium difficile* to hospitals, technology that could streamline the screening process and prevent many hospital-acquired infections

CUTTING DOWN THE testing time for certain medical conditions means care teams can more efficiently issue proper treatment to patients and mitigate the possibility of contamination. One species of bacteria, *Clostridium difficile* (C. diff), causes severe diarrhoea and other intestinal diseases, and has a high rate of contamination and recurrence in patients. The prevalence, spread and return of this disease can be diminished by more rapid diagnosis. The financial benefits of testing for this disease more quickly are also a motivation for developing more effective testing methods, as the cost of C. diff-associated disease (CDAD) on the healthcare system is US $1.1 billion annually and in Europe is estimated to be 3 billion euros per year. These expenses are expected to increase by as much as twofold over the next four decades.

CLOSTRIDIUM DIFFICILE

C. diff affects patients by causing symptoms ranging from diarrhoea to life-threatening colon inflammation. The disease is often prompted by the destruction of the normal gut flora by antibiotics, which provides C. diff with an ideal environment for it to thrive. Any instrument designed for detecting and diagnosing the bacteria must take into account that one of the strains of C. diff is a hyper-virulent strain associated with severe morbidity and mortality in patients. This alternative strain is caused by a mutation in the TcdC gene which results in an overexpression of the two toxins emitted by C. diff. "We intend to distinguish this strain from wildtype in order to enable optimum therapy and provide critical information to hospital administrators for mitigating further transmission," Irvine confirms.

A sensitive assay that is capable of processing and detecting C. diff spores would substantially protect the health of the patient as well as reduce the risk of spread of CDAD in hospitals, nursing homes, and rehabilitation facilities

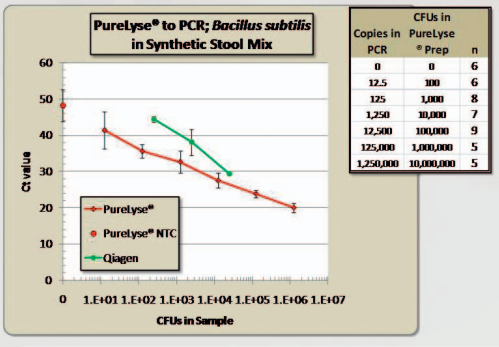
Most methods for extracting DNA for analysis require a lengthy process of up to a dozen steps and a minimum of one hour for completion. A form of medical testing known as point-of-care testing (POCT) can be conducted at or near the patient's bedside and significantly decreases the time it takes to reach a diagnosis. POCT brings the testing process instantly to the patient, thus reducing the need to send samples to a laboratory. This increases the likelihood that results will be available quicker, which in turn allows for clinical management decisions to be made on the spot.

A recent survey of healthcare professionals conducted as part of this project for developing a point-of-care technology for testing C. diff demonstrated a desire for such an instrument to be made available at nurses' stations. Bruce Irvine, Chief Technology Officer at Claremont BioSolutions LLC where the technology is being developed, explains the need for POTC technology: "Results were overwhelmingly in favour of a point-of-care instrument to be placed at nurses stations. This can obviate the need to send the samples to a centralised lab enabling healthcare workers to promptly issue proper treatment to patients and to mitigate contamination". The project team is working to create a low-cost system for analysing DNA in a 15 minute three-step process for detecting C. diff from patient stool samples and from swabs of medical devices.

Nearly every case of pseudomembranous colitis is brought on by the C. diff bacteria as well as up to 25 per cent of antibiotic-associated diarrhoea. Cases of C. diff infection typically recur in a third or more of patients within the first month. Furthermore, the bacteria can remain hidden in a person's gut if the prescribed antibiotic treatment is insufficient and reappear weeks or months later to cause a second round of infection.

Another major reason for the high rate of recurrence and prevalence of C. diff infection is

FIGURE 1. Detection of Bacillus subtilis in Synthetic Stool Mix: PureLyse® sample prep to PCR.



effectively, thus reducing the conversion to the active form. Therefore, patients infected with the highly virulent strains may benefit from an immediate treatment with oral vancomycin.

Could you describe the form and function of the unique technology, PureLyse (PL), that you have developed?

PL is a miniature, disposable flow-through cartridge packed with 100 uM Zirconium/silica beads (~80 per cent/20 per cent) that are used to mechanically lyse the cells and, under the specific conditions, bind and release DNA. PL contains a lead-free micro-motor equipped with a precision-cut impeller.

A proprietary method is used for entrapping and retaining the beads within the lysis chamber during the activation of the motor up to 30,000 rpm. The shear forces generated are sufficient to lyse thick-walled cells, bacterial spores, and Giardia cysts. Also unique is the PL buffer formulation employing pH rather than guanidine/ethanol to mediate binding of DNA to the Zr/Si beads. This minimises the requirement for onerous washing to remove these polymerase inhibitors.

We have also embedded the PL chamber within a fluidic valve with up to six ports. Combining this valve with a reversible pump capability has enabled intricate fluid management within a simple architecture.

A FULLY INTEGRATED ASSAY AND PLATFORM FOR DETECTING CLOSTRIDIUM DIFFICILE

OBJECTIVES

We will develop a point-of-care (POC) instrument that will extract DNA from stool samples and environmental swabs to detect Clostridium difficile within 15 to 20 minutes. We integrate our unique PureLyse® sample preparation method with a real time isothermal amplification method into a disposable cartridge leading to a handheld device.

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BRUCE IRVINE is currently engaged as Chief Technology Officer developing and commercialising systems for sample preparation in processing nucleic acids and proteins for research and clinical applications. His previous experience includes managing Research and Development at Clinical MicroSensors (GenMark) and Chiron Diagnostics (Siemens), developing clinical assays for infectious diseases, such as HBV, HCV, HIV and genetic disorders such as cystic fibrosis and Cytochrome p450 liver enzymes.

Prototype of integrating the Purelyse® chamber with the Omnivalve™ and two reagent vessels.

that it is spore-forming and can contaminate surfaces for several months, resisting most types of disinfectants. C. diff has risen to be the most common source of hospital acquired infections in the U.S. with 3 million cases reported each year. Most of the deaths related to C. diff occur among older people. The number of deaths resulting from infection reached their highest in 2007 with over 8,000 incidents.

Irvine outline the urgent need for improved testing methods: "There is clearly a need for a reliable, sensitive, and expeditious method for testing of environmental contamination. A sensitive assay that is capable of processing and detecting C. diff spores would substantially protect the health of the patient as well as reduce the risk of spread of CDAD in hospitals, nursing homes, and rehabilitation facilities".

DETECTION TECHNOLOGY

Staff at Claremont BioSolutions are currently in their final year of Phase I of their project to develop the very first POCT system for detecting C. diff. At the heart of the project is the company's innovative PureLyse® (PL) device, miniature technology that condenses ultrafast mechanical cell lysis and extraction of genomic DNA into a three step protocol that is both cost- and time-efficient.

Currently, the most widely used tests to detect the presence of C. diff are enzyme immunoassays (EIA). To perform an EIA, an unspecified amount of antigen is attached to a surface and a particular antibody is placed over it for binding. The antibody will have a connection to an enzyme which can often be detected by a colour change in a chemical substrate in the final step of the process. These tests yield results quickly, but a major downside is their lack of sensitivity. It is a difficult balance

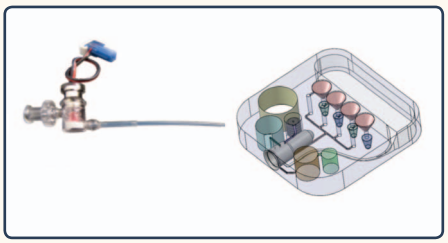

FIGURE 2. Functional decomposition into sample preparation unit and amplification/detection. The four optically clear reaction chambers extent below the cartridge to fit into the fluorometer wells.

to strike between speed and accuracy, as the more sensitive tests, such as cytotoxicity assays can take several days to complete.

The new technology is expected to take the form of a handheld device that is simple, self-contained and does not require specific skills to operate. "The greatest challenge," Irvine admits, "is making the system foolproof, so that it gives the unskilled user the ability to get an accurate answer and does not introduce a new risk of contamination." He expects the instrument to provide an accurate reading of any contamination and provide care staff with an answer in less than 20 minutes from the time the swab is taken. This faster turnaround rate will support better therapy and mitigation response.

AN INTEGRATED ASSAY

Now in the final year of its Phase I funding, the project team has acquired strong data using their unique technology on synthetic stool mixes. Irvine expects the first tests using real stool samples to be performed in the near future and to be able to produce and deploy POCT for C. diff to hospitals in the next four to five years.

Irvine and his group have already applied the NEAR assay on cultured C. diff. This assay, which is able to magnify low amounts of starting material to easier levels of detectability, is considered to be the best choice for POCT thanks to its superior speed and sensitivity. The successful development of a sensitive assay that has the power to process and detect C. diff spores in a matter of a few minutes would transform the contemporary approach to detecting the bacteria, prevent its outbreak, and effectively slash the weight it places on the healthcare system.