An automated in-situ biosensor for quasi real-time detection of coliform bacteria in public waters

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Introduction

Background: One of the holy grails of water quality testing is the ability to monitor water quality in a rapid, real-time manner. The ability to decrease the time to obtain water quality results depends on a few things, among them the development of new assays that give results in as fast a manner as possible. Another way to decrease the time to result would be to miniaturize and automate commercially available assays in order to reduce the required sample size, reduce the need for operator expertise and reduce assay time, reagents, and cost. For example, the typical protocol for testing water requires acquiring a sample, transporting it (in cold) to a lab, performing bacterial enrichment, performing the assay and communicating the results. Depending on the method used, this entire process can take days or even weeks to obtain results. The biological assay itself may be a small part of the entire process. This project proposes to eliminate the manual sample acquisition and transportation steps, automating the enrichment and assay steps and automate the result communication step by building a platform capable of performing a water quality assay *in-situ* and having a computer communicate the results to the could where they are accessible to the public.

Previous Work

Detection of ESKAPE bacteria using isothermal DNA-based assays in a portable, de-gas microfluidic diagnostic platform. A current technology gap associated with available diagnostic tests is the inability to prevent over prescription of antibiotics to patients, and the trial-and-error approach of treatments, which increases risk for patients, reduces the lifetime of antibiotics, and contributes to antimicrobial drug resistance. Our work addresses this gap by developing a rapid, inexpensive, portable, and easy-to-use microfluidic system for detecting bacterial pathogens that are most commonly associated with antibiotic resistance and are known as the ESKAPE bacteria: Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp. We use a vacuum-degassed PDMS device pre-loaded with recombinase polymerase amplification (RPA) assay reagents to detect five of the six ESKAPE pathogens with high sensitivity (e.g., a limit of detection of ~10 nucleic acid molecules) that is comparable to bench-top PCR-based assays, and a small, portable battery-powered electronic incubator/reader that enables these assays to be performed at the point-of-care.

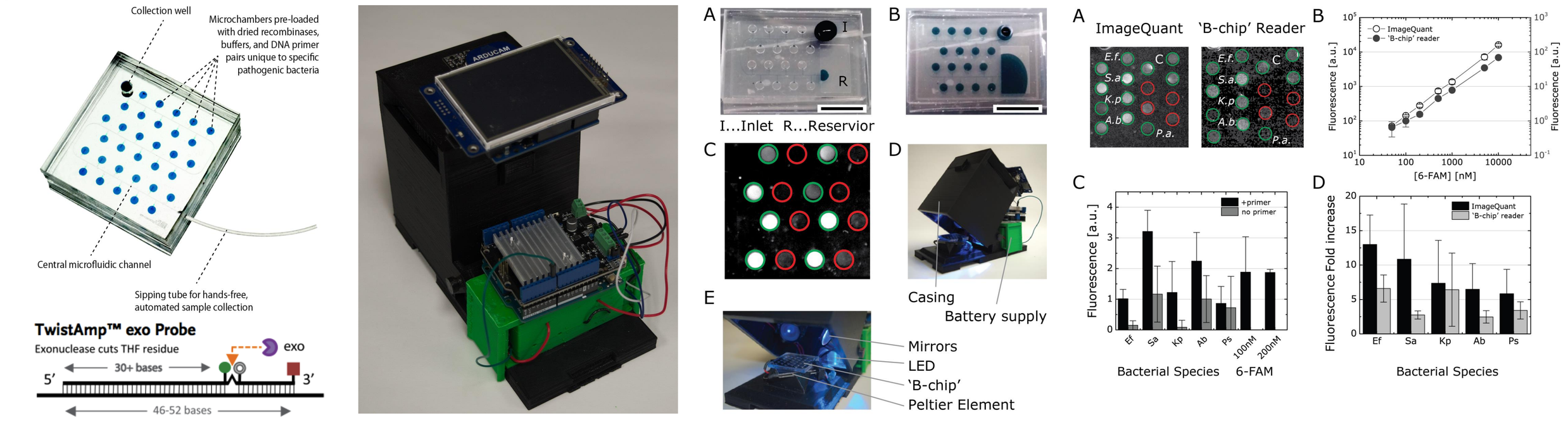
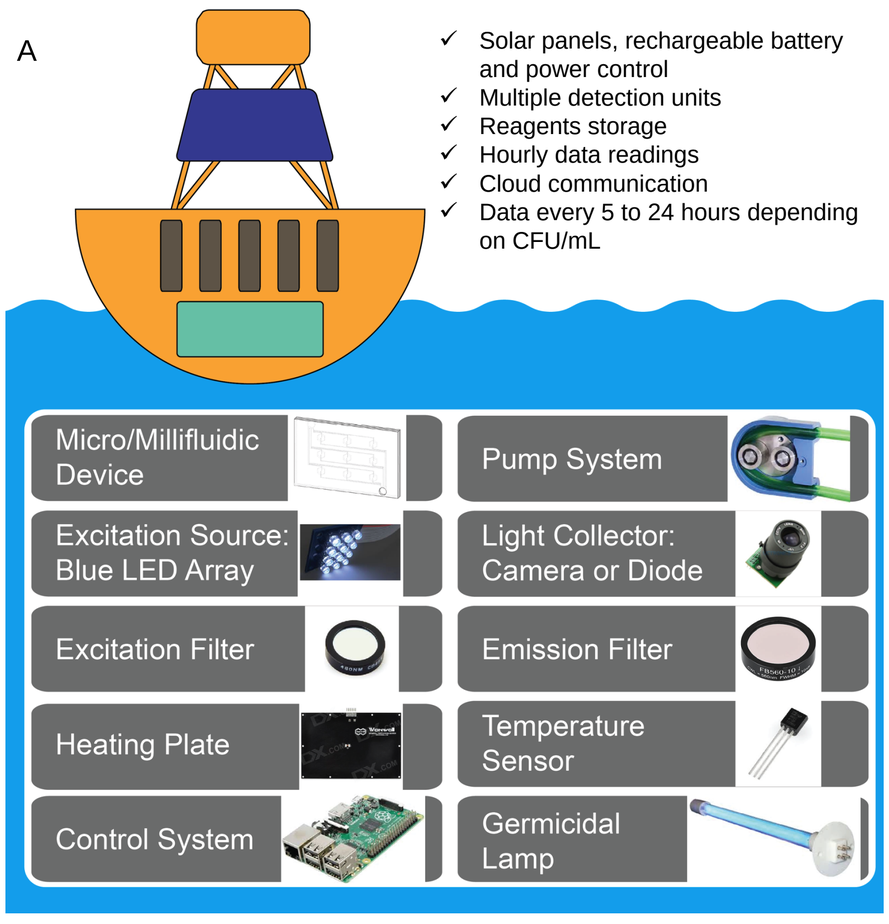


Figure 1. Point-of-Care DNA detection system. *Left*: Self-loading PDMS device using RPA for genetic amplification and fluorescent exo-probes for identification. *Center left*: Custom-made portable battery-powered electronic incubator/reader capable of detecting positive RPA assay results. *Center right*: A,B) Empty and loaded PDMS device. C) Comparison of primer versus no primer RPA results. D,E) PDMS device inside electronic incubator/reader . *Right:* A,B) Fluorescent data comparison between GE ImageQuant and electronic incubator/reader. C) Fluorescence intensity of RPA assay using +primer versus no primer samples. D) Fluorescence fold increase of RPA measured with GE ImageQuant and electronic incubator/reader for different bacterial species.

Current and Future Work

Development of first generation in-situ biosensor for quasi-real time detection of fecal indicator bacteria: We propose to develop an automated quasi-real time *in-situ* biosensor for detection of coliform bacteria in small water sample volumes using commercially available FDA approved bio-detection assays. The main components of this system are a biological assay, pumps and valves, fluorescence excitation and emission filters, a temperature incubator, and data acquisition and analysis systems. As the biological assay incubates it gets excited by a light emitting diode (LED), the scattered fluorescence is detected by an optical sensor that will quantify the amount of coliform bacteria. The system will be controlled by a micro-computer and a microcontroller. This system will have 3G or Wi-Fi capabilities to send data to the internet. Each sensor (measuring samples in the milliliter range) will be capable of producing a result every 5 to 24 hours, depending on the amount of coliform bacteria present. This reduces the current processing time and increases the current testing throughput. The first generation of the biosensor will be a bench top unit capable of performing the necessary steps for automated *E. coli* or *Enterococcus* detection.



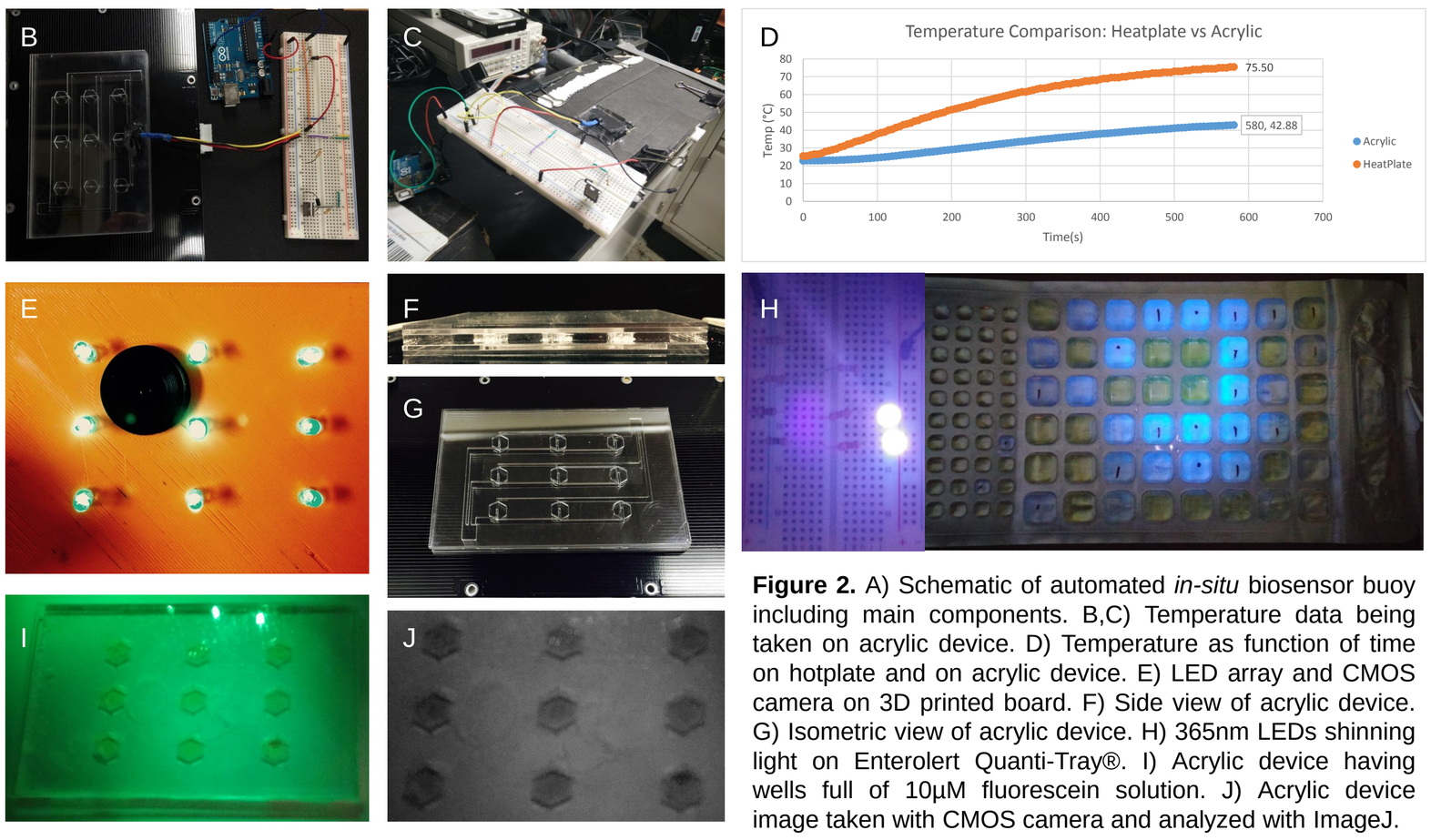


Figure 2. A) Schematic of automated *in-situ* biosensor buoy including main components. B,C) Temperature data being taken on acrylic device. D) Temperature as function of time on hotplate and on acrylic device. E) LED array and CMOS camera on 3D printed board. F) Side view of acrylic device. G) Isometric view of acrylic device. H) 365nm LEDs shinning light on Enterolert Quanti-Tray®. I) Acrylic device having wells full of 10µM fluorescein solution. J) Acrylic device image taken with CMOS camera and analyzed with ImageJ.

References and Acknowledgements

Enterolert and Quanti-Tray® system are registered trademarks of IDEXX Laboratories Inc., Maine USA.
"Detection of ESKAPE bacteria using isothermal DNA-based assays in a portable, de-gas microfluidic diagnostic platform" project was sponsored by the Bill & Melinda Gated Foundation and is the work of the following authors: Lars D. Renner, Jindong Zan, Linda I. Hu, Manuel Martinez, Pedro J. Resto, Adam C. Siegel, Clint Torres, Sara B. Hall, Tom R. Slezak, Tuan Nguyen, Douglas B. Weibel.
Funding for this project provided by CariCOOS. Future work in this project will be sponsored by CariCOOS and Sea Grant project number R/92-02-16.