

E.coREADi: Automated Microbiological Water Analysis

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LUNA

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INTRODUCTION

The Environmental Protection Agency (EPA) Office of Water oversees the implementation of the Clean Water Act and Safe Drinking Water Act in fulfilling its mission to protect human health and the environment. Identifying microbiological contamination is critical to ensure the safety of potable and recreational waters. *E. coli* and coliform bacteria are routinely measured, as their presence indicates an increased likelihood of pathogens due to their fecal origin. EPA approved analytical methods for microbiological water analysis require a laboratory, technical expertise, and a minimum time requirement of 18-24 hours. Technologies that enhance compliance monitoring by reducing the analysis time, operator requirements, or enabling use by non-experts will therefore provide a substantial benefit to end-users and customers. The ability to detect *E. coli* and coliform bacteria in near real-time has also been identified as critical to mission success by the US military.

To meet this need, Luna Innovations is developing the E.coREADi™ technology for the automated analysis of *E. coli* and total coliform bacteria within drinking water. E.coREADi automation leverages prior development of the E.coREADi rapid assay kit to facilitate testing of wastewater effluents aboard US Navy vessels and ensure EPA compliance prior to discharging treated water. The assay kit enables detection of viable *E. coli* with a proprietary luminescent enzyme-substrate based system, and requires approximately 15-30 minutes of hands-on time for end-users, with a 10 hour total assay time.

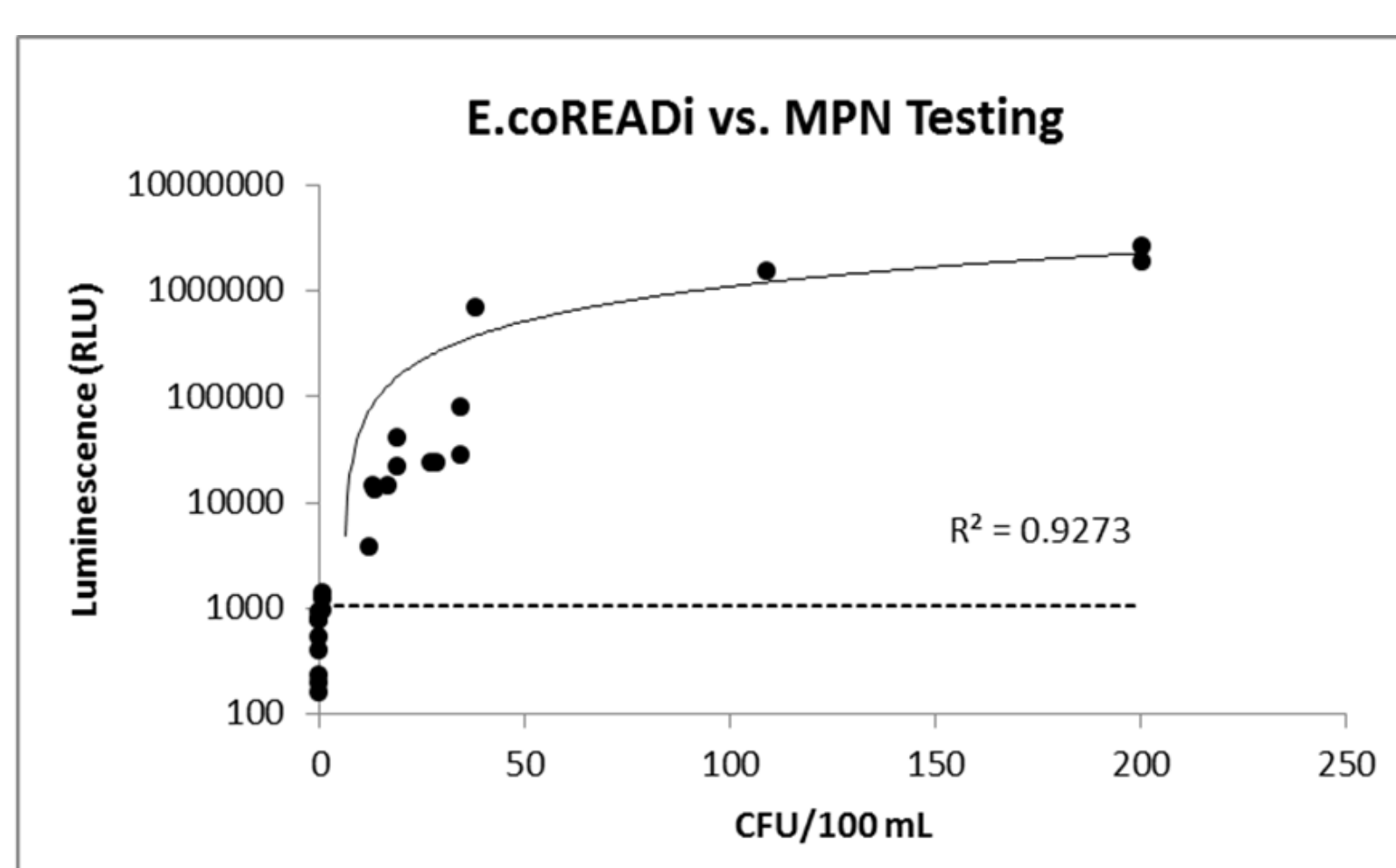


The E.coREADi technology was developed for the rapid and quantitative detection of viable *E. coli* in wastewater effluent aboard US Navy vessels.

OBJECTIVES

The E.coREADi assay is currently being refined for drinking water analysis and to meet the operational requirements of the US Army. This includes:

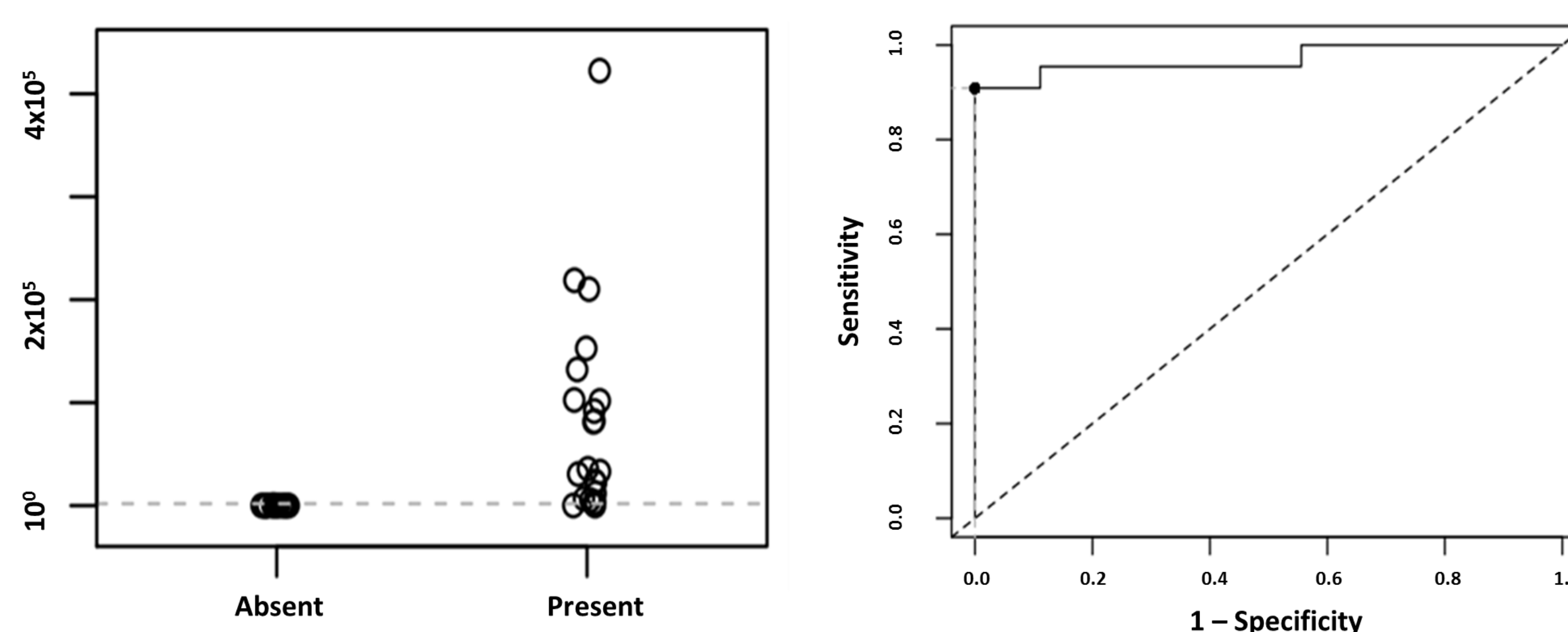
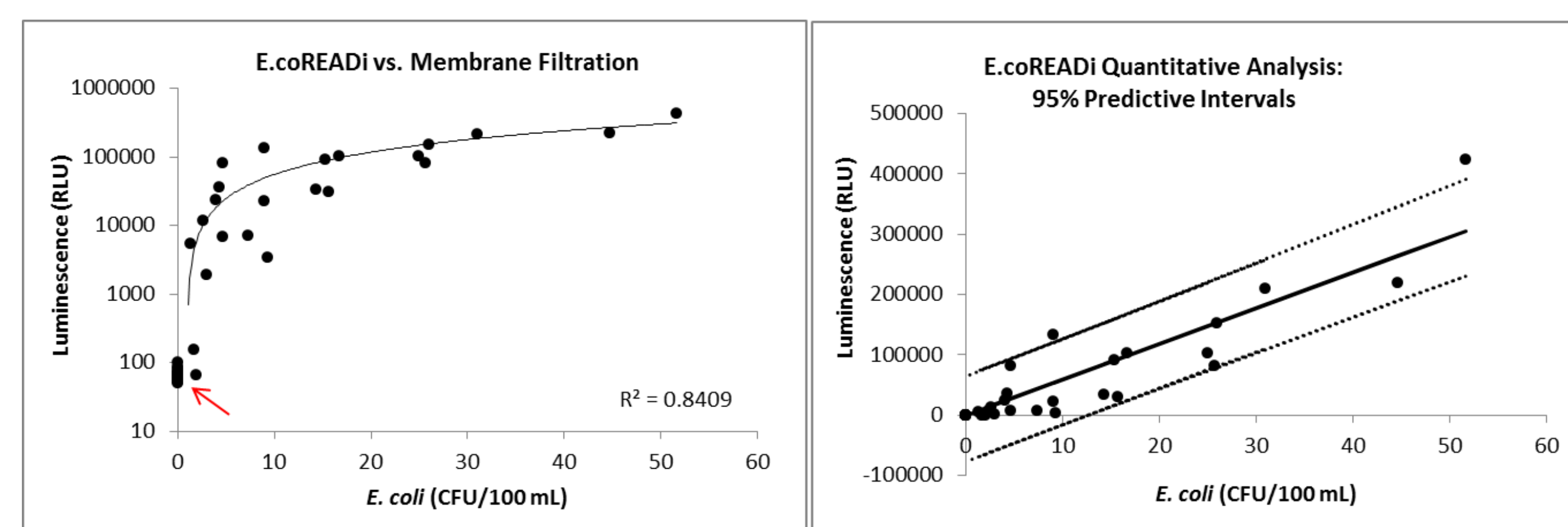
- Demonstrating rapid recovery of chlorine-stressed organisms.
- Refining reagents and protocols for detection of both *E. coli* and total coliforms.
- Integrating presence/absence detection methods (<1 CFU/100 mL).
- Transitioning the assay to an automated microfluidic cartridge format.
- Developing an E.coREADi instrument to control the cartridge, analyze results, and provide complete automation for the end-user.



E.coREADi assay agreement with Colilert QuantiTray Most Probable Number (MPN) methods. Independent testing was performed by collaborators with multiple wastewater effluents over 10 independent test days. All data points are averaged from 3 E.coREADi and MPN replicates.

E.coREADi ADVANTAGES

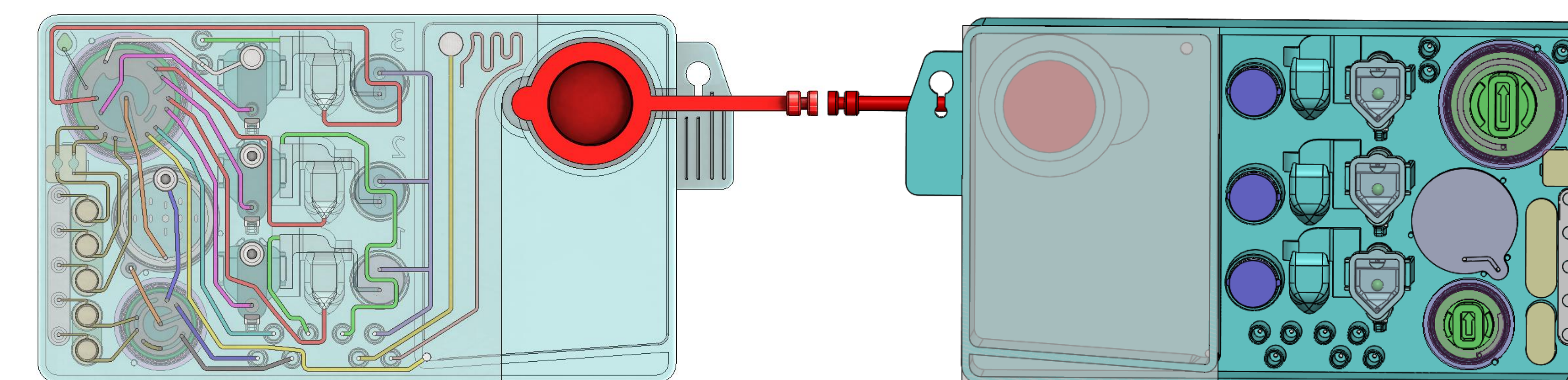
- **Sensitivity and specificity:** Luna has demonstrated presence/absence detection of chlorine-stressed organisms within a total assay time of 10 hours. E.coREADi growth media is formulated to selectively enrich target bacteria, and enzyme-substrate methods provide additional specificity in the detection of *E. coli* and total coliforms.
- **Microfluidic format:** Microfluidics enable transition of hands-on laboratory assays requiring technical expertise to an automated and user-friendly format. E.coREADi microfluidic cartridges are low-cost injection-molded plastics designed for single use.
- **Automated detection:** Automated analysis reduces user-error and hands-on operator requirements (<5 minutes). Simply fill the self-metered (100 mL) water reservoir, insert the cartridge to the instrument, and receive your results 10 hours later.
- **Same-day customer results:** The E.coREADi instrument will be designed to send results to end-users and customers via text or email, offering a same-day result without burdening laboratory operators with long days or results analysis requirements.



E.coREADi assays were performed with wastewater effluent over 6 independent days of testing with multiple effluents. Results are presented on a logarithmic y-axis (top left) to discriminate detection of low numbers of *E. coli* and on a standard axis with prediction intervals (95%, dashed lines) for integration of quantitative standards (top right). Presence/absence capabilities were demonstrated using Receiver Operator Characteristic (ROC) curve analysis to identify the optimal signal cutoff with respect to sensitivity and specificity (bottom left). Assay sensitivity, specificity, and accuracy was determined to be 91%, 100%, and 97%, respectively, with ROC analysis (bottom right). All data points are averaged from 3 E.coREADi and membrane filtration replicates.

E.coREADi AUTOMATION

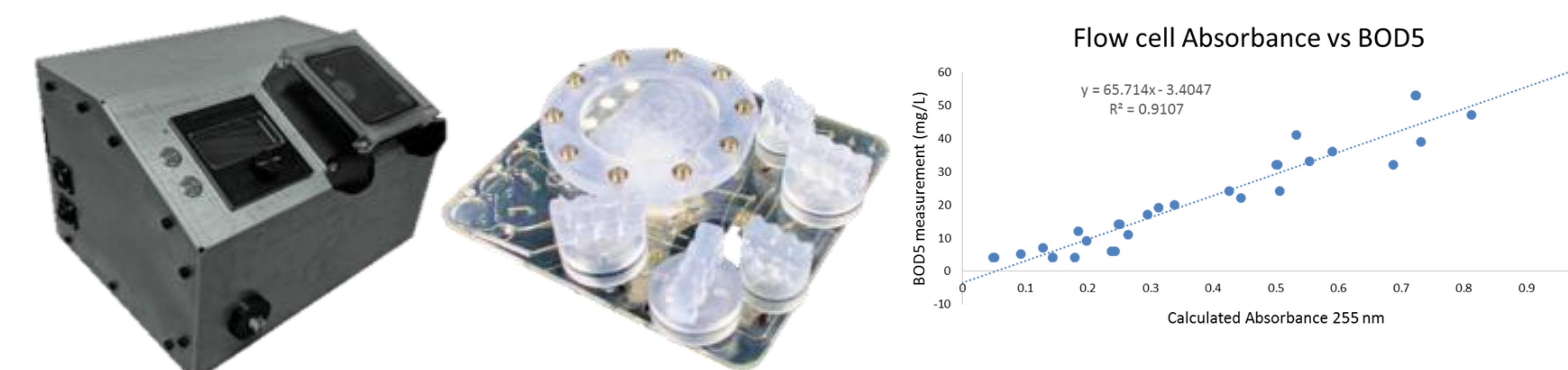
The E.coREADi cartridge has been designed, prototyped, and manufactured with partners at Symbient Product Development. Cartridges integrate all features for assay automation, including a self-metering 100 mL sample reservoir, shelf-stable lyophilized reagents and reconstitution buffers, and elements for metering, mixing, and detection. The E.coREADi instrument interfaces with the cartridge through liquid, pneumatic, and mechanical actuation. Luna is currently assembling system prototypes that integrates all elements for actuation, incubation, and high sensitivity optical detection. The instrument will be low-footprint, portable, battery-operable, and allow analysis of up to 16 samples simultaneously.



The E.coREADi microfluidic assay cartridge is a single-use disposable assay cassette.

RELATED WORK

The E.coREADi technology has also been integrated within the AWQuA-MD™ system, a total water analysis suite. This in-line and automated system was developed to enable detection of *E. coli*, biological oxygen demand, and total organic carbon for the US Army. For this technology, Luna worked with Symbient PD to integrate the E.coREADi into injection molded microfluidic cartridge format to reduce reagent and consumables cost. This format requires the end-user to add reagents and sample to the cartridge, with a custom instrument developed at Luna that automates the mechanical and pneumatic actuation of the assay on-board.



The AWQuA-MD system integrates the E.coREADi assay into an automated microfluidic format for *E. coli* detection in wastewaters, with in-line optical measurements for determining biological oxygen demand (BOD5) and total organic carbon. The prototype system and cartridge are shown, as well as flow-cell measurements of wastewaters over 6 weeks plotted against traditional BOD5 analyses.

CONCLUSION

To date, Luna has demonstrated presence/absence detection capabilities for viable *E. coli* and total coliforms in 10 hours with chlorine-stressed bacteria using the E.coREADi method. Prototype systems and microfluidic cassettes are currently being manufactured for final validation work prior to submission of a study plan for EPA Alternative Test Protocol (ATP) testing and approval. With complete automation, rapidity, and minimal operator training requirements, the E.coREADi technology is anticipated to have private sector applications in municipal water facilities, environmental testing laboratories, disaster relief missions, and food and beverage manufacturing facilities.

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LUNA'S HEALTH SCIENCES GROUP

Luna Innovations Incorporated (NASDAQ: LUNA) develops and manufactures next-generation products for the healthcare, telecommunications, energy and defense markets. Luna's HEALTH SCIENCES GROUP is an interdisciplinary team of biologists, bioengineers, and materials scientists who conduct research and development in wound healing, medical devices, drug delivery, medical simulation, and diagnostics. For more information on the Health Sciences Group, please contact Dr. Christopher Tison, Director, tisonc@lunainc.com