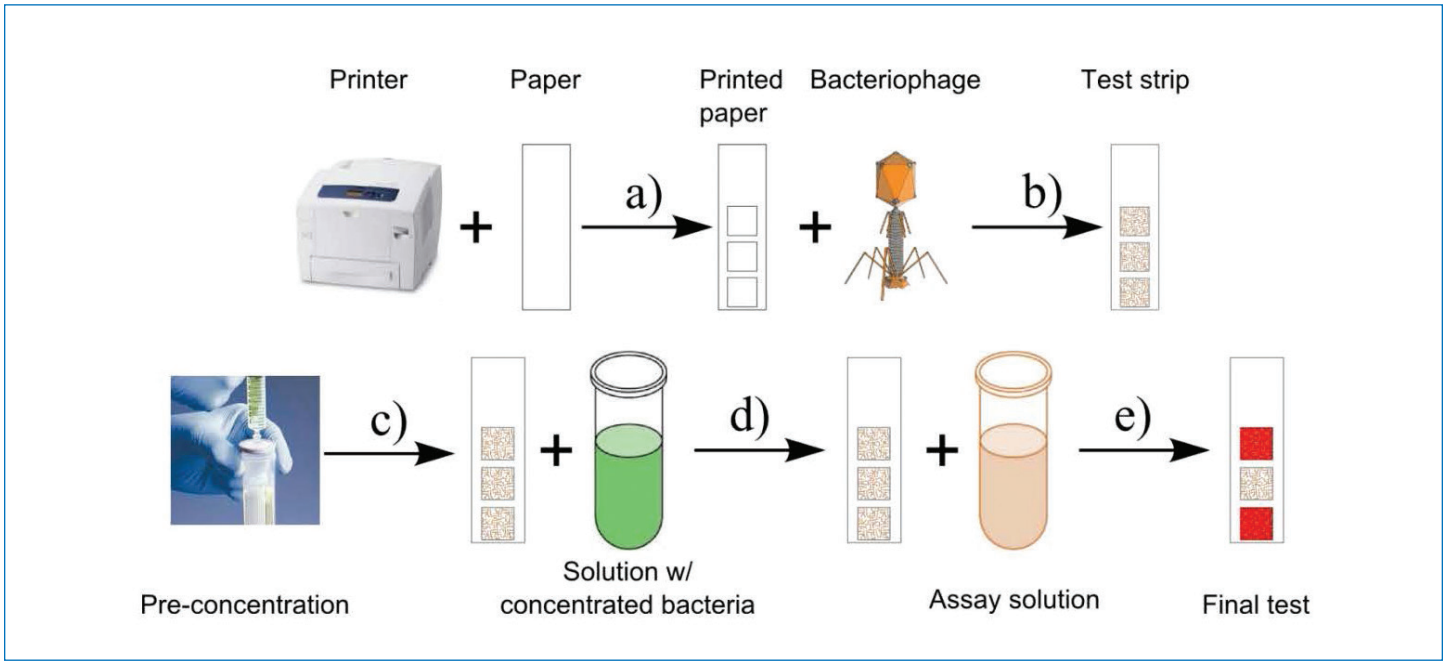
Executive **Summary**

Water quality monitoring at beaches with results in under two hours

Simple and Fast Detection of *Escherichia coli* in Recreational Waters (U4R13)



Detection of bacteria with a bacteriophage containing dipstick test.
a) Paper is patterned. b) Bacteriophage are bound. c) Sample is pre-concentrated. d) E. coli are bound to dipstick. e) E. coli are exposed to assay.

The Central Issue

Until recently, the U.S. EPA's recommended water quality testing strategies were dependent upon culture-based methods, which require one to two days before results are available. During that time, recreationists could potentially be at risk (Noble and Weisberg, 2005). Indicator organisms, such as *E. coli* and enterococci, are used to determine when a risk to human health is present. As the concentrations of indicator organisms increase, it is likely that pathogenic organisms present in fecal material will also be in the waterbody, presenting a risk to human health (U.S. EPA, 2002).

Today, detection of pathogens and pathogen indicators via monitoring can also employ enterococci as determined by quantification through polymerase chain reaction (qPCR). While qPCR is promising due to the rapid turnaround time, analytical costs are high and specialized laboratory equipment is required.

It is routine to use quick and low-cost based diagnostics in water testing for contaminants like nitrate and phosphate. The development of a paper-based device for detection of water-borne pathogen indicators has the potential to transform current recreational water quality monitoring programs by allowing testing at the point-of-contamination with results in hours instead of days.

Context and Background

The researchers in this study wanted to develop a simple dipstick-type test for the detection of fecal indicator bacteria (FIB) *E. coli* in recreational waters that would provide results in less than eight hours. The expected deliverables related to achieving this objective were:

- Bind bacteriophages to paper.
- Pattern paper to create a dipstick-type test.
- Design a quantitative colorimetric assay for bacteria on paper.
- Develop a pre-concentration device for use in the field.
- Design an *E. coli* field detection kit.
- Field test the bacteria detection kit and compare results to standard EPA detection methods.*

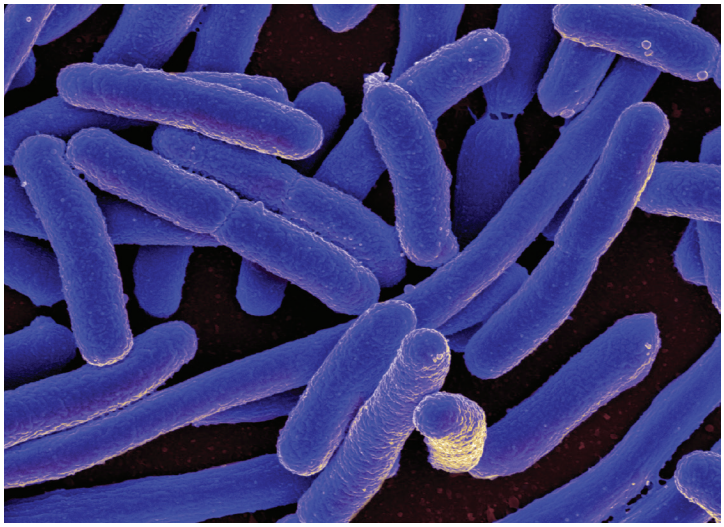
Findings and Conclusions

This new testing methodology combined the availability and low-cost of paper with the specificity of bacteriophages into a device that captured *E. coli* in five minutes or less. After capture of the *E. coli*, enzymatic assays detected the presence of the bacteria on the paper by a color change. This device detected 10^5 *E. coli* in spiked lake water per

100 ml in one hour. It was possible to detect *E. coli* and their metabolic state in less than two hours using the paper-based assay developed in this research. It was significantly lower than the eight hours needed for EPA standard methods. The researchers developed all necessary components to test for *E. coli* directly in the field. Note however, that low contamination in the lakes selected for the study during the summer of 2016 and a limit of detection that was too high to detect *E. coli* without pre-concentration prevented the field-testing portion of the research.* The components now need to be combined and field tested.

Management and Policy Implications

This new testing methodology allowed the testing of water samples for beach monitoring in less than two hours with a potential to do testing at the site of contamination. It drastically decreases the turnaround time for the results and reduces the risk of water-based bacterial infections with more timely closures of affected beaches. Moreover, the elimination of sample transportation to laboratories for analysis saves the cost of transportation as well as reduces the carbon footprint.



Colorized scanning electron micrograph of Escherichia coli.

Source: www.flickr.com/photos/tags/nationalinstituteofallergyandinfectiousdiseases

Related WE&RF Research

Project Title and Number	Research Focus
White Paper on the Application of Molecular, Spectroscopic, and Other Novel Methods to Monitor Pathogens for Potable Reuse (Reuse-14-17)	Documents the current state-of-the-science on molecular characterization methods, such as qPCR, RT-qPCR, next-generation DNA sequencing or pyrosequencing, nucleic acid sequence based amplification (NASBA), and droplet digital PCR and other novel methods.
Validation of Rapid Methods for Enumeration of Markers for Human Sewage Contamination in Recreational Waters (PATH3C09)	Compares culture-based measures of the fecal indicator bacteria to rapid quantitative polymerase chain reaction (qPCR) methods in terms of correlation and consistency of performance in various types of subtropical waters in Florida.
Quantification of Pathogens and Sources of Microbial Indicators for QMRA in Recreational Waters (PATH2R08)	Synthesizes existing information and generated new data about relationships between pathogen indicators, pathogens, and source identifiers to develop a quantitative microbial risk assessment (QMRA) framework for recreational waters.
Comparative Evaluation of Molecular and Culture Methods for Fecal Indicator Bacteria for Use in Inland Recreational Waters (PATH7R09)	Provides guidance on where quantitative polymerase chain reaction (qPCR) may be successfully applied for monitoring of pathogen indicators, how much variability in measurement may be anticipated, assessment of inhibition, and its relationship to culture-based assays.
Molecular Methods for Measuring Pathogen Viability/ Infectivity (Reuse-15-07) (ongoing)	Seeks to develop a new or improved method for measuring infectious/viable pathogens in sewage, treated wastewater, and reuse water, including that from indirect potable reuse (IPR) and direct potable reuse (DPR). The new or improved method must result in faster turnaround times for concentration and analysis, in greater method sensitivity, and reduce overall method costs, relative to current methods.

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