Enhancing the Value of Molecular Methods to the Water Industry: An *E. Coli* Case Study [Project #4238]

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OBJECTIVES

The goal of WRF Project 4238 was to collaborate with water utility laboratories to evaluate whether molecular testing can be of value for monitoring water quality associated with their operations, with a specific focus on detection of viable *E. coli* in source and finished drinking water.

BACKGROUND

The standard methodology for total coliform and *E. coli* testing by water utilities is culture using agar or broth media. Increasingly, molecular techniques—such as PCR and RT-PCR—have become standard, well-established tools for detecting and quantifying the presence of microbes in clinical, environmental, and food samples. Molecular testing for *E. coli* may also prove to be a valuable tool for water utilities, either alone or in conjunction with standardized culture methods. Unlike culture methods that often require incubation for 18–24 hours, molecular testing techniques have the potential to detect and confirm the presence of *E. coli* on the same day as sample collection, thereby providing water quality data with shorter turnaround times for water utility use. However, no standardized methods for molecular analysis of water samples have been reported for use by water utilities or environmental scientists. A multitude of alternative methods, supplies, and equipment are available, but little evidence-supported guidance is available that water laboratory professionals can use to compare alternative methods, optimize a selected method, establish performance expectations, account for method inhibition, and troubleshoot assays when faced with unexpected results.

APPROACH

A literature review was conducted to identify alternative sample processing and molecular assay techniques for *E. coli*. Several DNA and RNA molecular assays were screened to identify the best assays for further performance evaluation. Selected molecular assays were optimized and standardized according to EPA QA/QC guidance for PCR. Additionally, an

internal control was optimized and standardized for quality control and potentially to serve as a method for assessing sample inhibition. Two sample processing approaches, a culture-dependent method (RC-PCR) and a culture-independent method (PMA-PCR), were investigated for performance in conjunction with the developed molecular method with the goal of enabling molecular analysis to meet current regulatory requirements for detecting viable *E. coli* in drinking water. The RC-PCR method was compared with several EPA-approved culture-based methods to evaluate differences in sensitivity and specificity. This method was transferred to participating water utilities for their comparison with culture-based *E. coli* methods. An inter-laboratory study was conducted in which each participating laboratory performed the RC-PCR method in parallel with an EPA-approved culture technique for at least 5 water samples on a weekly basis for 6 months.

RESULTS/CONCLUSIONS

The literature review identified many candidate gene targets and molecular assays for screening. In total, 18 molecular assays were evaluated, including published assays and assays developed by CDC for this project. Selected assays were optimized to identify the assay or combination of assays that provided the most sensitive and specific detection of *E. coli*. It was determined that a multiplex TaqMan assay targeting *uid*A (beta-glucuronidase) and *tna*A (tryptophanase operon) with an internal control provided excellent sensitivity and specificity. The culture-dependent sample processing method paired with the multiplex PCR assay (RC-PCR method) was found to provide similar sensitivity and specificity as several EPA-approved culture methods. By combining a short-term (5-hour) culture step with a molecular assay, the RC-PCR method has the potential to detect viable *E. coli* within an 8-hour working day. The culture-independent sample processing method paired with the multiplex PCR assay (PMA-PCR) could detect viable *E. coli* without culture using propidium monoazide (PMA), however the method detection limit was relatively high (50 CFU/100 mL). The multiplex PCR assay was also used to confirm the results of EPA-approved culture methods by analyzing colony material or broth culture. The results of the inter-laboratory validation study showed that the RC-PCR method was comparable to EPA-approved culture methods for *E. coli* detection in source water, finished drinking water, and distribution system water.

APPLICATIONS/RECOMMENDATIONS

The molecular methods developed in this project have the potential to be useful to water utilities for detecting the presence of viable *E. coli* in their water systems within a shorter time frame (8 hours) than possible using EPA-approved culture methods (18–24 hours). The multiplex-PCR assay is also potentially useful as a technique for confirming the results of standard culture-based tests. However, feedback from utility collaborators indicated that the utility of the RC-PCR method for *E. coli* detection would likely be limited to a narrow set of applications. These applications included emergency response (when fecal contamination is known or suspected) and when finished water samples can be collected early in the work day. These utility collaborators indicated that the utility of the method was limited because most samples do not arrive in the lab for testing until the afternoon, leaving insufficient time for the 8-hour RC-PCR method. Holding samples overnight for processing by RC-PCR the next morning would yield little advantage over traditional culture methods. In addition, utility collaborators

indicated that the method was labor-intensive compared to traditional culture methods. They also stated that data from such molecular methods for *E. coli* would have limited value because the data could not be used within the current regulatory framework. It would therefore be useful for EPA to evaluate the RC-PCR method (or alternative molecular techniques) for use by water utilities to produce water quality data that can be used for regulated water quality monitoring and/or confirmation of standard culture-based results.

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