

## Project Summary

### A Comparison of Ten USEPA-Approved Enzyme-Based Total Coliform/*E. coli* Tests for Microbiological Groundwater Monitoring and Laboratory Consultation WR04R003

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**Period of Contract:** 6/30/03 – 6/30/05

**Background/Need:** Protection of groundwater from microbial contamination is a top public health priority. Recent epidemiological studies clearly show that gastrointestinal disease due to ingestion of drinking water is occurring at significant levels in the United States and Canada(1). Furthermore, the United States Centers for Disease Control reported in their last 10 year summary of waterborne disease outbreaks that over 70% of the documented outbreaks occurring in the U.S. were associated with contaminated well water (2). These facts underscore the need for sensitive, reliable laboratory methods to identify microbial contamination in groundwater that might pose a potential risk of illness. Since 2002, the United States Environmental Protection Agency (USEPA) has approved ten enzyme-based total coliform and *E. coli* detection tests for examination of drinking water. Differences in the ability of some of these methods to detect total coliform and *E. coli*, as well as suppress *Aeromonas spp.*, a common cause of “false positive” results, have been observed. As a result, this study was undertaken to elucidate the strengths and weaknesses of each method.

**Objectives:** The objectives of the project were threefold; 1) to determine the capabilities of all of the USEPA approved products to detect the presence or absence of total coliform and *E. coli* in three chemically diverse groundwaters, 2) to determine the ability of each product to accurately quantify the number of total coliforms and *E. coli* in groundwaters, 3) to determine each product’s ability to suppress various concentrations of *Aeromonas spp.*, which represent a non-coliform, heterotrophic bacteria likely to occur as a false positive interference (14,15).

**Methods:** Water samples were collected from three geographically and chemically diverse groundwaters in Wisconsin. One-hundred milliliter aliquots were individually spiked with both low concentrations (one to ten organisms) and high concentrations (fifty to one-hundred) of each of five different total coliform organisms (*Serratia*, *Citrobacter*, *Enterobacter*, *E. coli*, & *Klebsiella*). These spiked samples were used to test the capability of ten enzyme based test systems to both detect and enumerate the spiked organisms. In addition, 100mL samples were independently spiked with two different strains of *Aeromonas spp.* at six different levels, to assess the ability of each enzyme-based test to suppress *Aeromonas spp.* Analysis of the data indicated that wide variability exists among USEPA approved tests to detect and quantify total coliforms, as well as suppress *Aeromonas spp.*

**Results and Discussion:** The data produced in this study suggests that there are significant differences between the ten USEPA approved methods both in the ability to detect/enumerate total coliforms and *E. coli* and in their ability to suppress false positive results from the non- coliform organism, *Aeromonas*.

Furthermore, this study demonstrates performance differences attributable to sample matrix differences. Some of the methods evaluated were unable to detect certain species of total coliform in some of the groundwater matrices examined. The most significant of these findings is the inability to detect *E. coli* even in high concentrations with some test method/sample matrix combinations. The site 3 groundwater characterized by a high level of background heterotrophic bacteria, low pH and low alkalinity (Table 1) was the most problematic.

**Conclusions/Implications/Recommendations:** Although the interaction of these parameters with test performance is not entirely understood, the author speculates that low pH and low alkalinity level samples such as the site 3 water may require a media formulation with greater buffering capacity. The data suggests the possibility that the Colisure and mColiBlue24 may not provide enough acid-neutralizing capacity to provide accurate results whereas the other products were capable of maintaining their integrity and efficacy in the water samples exhibiting these characteristics. Another possible explanation would be associated with the high level of background bacterial contamination.

**Related Publications:**

Proceedings of the American Society for Microbiology, Atlanta, Georgia, June, 2005, Proceedings of American Water Works Association Water Quality Technology Conference, Quebec City, Quebec, November 5-9, 2005.

J. Olstadt, J. J. Schauer, J. Standridge, and S. Kluender. A Comparison of Ten US EPA Approved Total Coliform/*E. coli* Tests. Submitted to *Journal of Water and Health*.

**Key Words:** total coliform, *E. coli*, enzyme-based methods

**Funding:** University of Wisconsin Water Resources Institute, Groundwater Research Projects