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A COMPARISON OF TEN USEPA-APPROVED ENZYME-BASED TOTAL COLIFORM/E. COLI TESTS FOR MICROBIOLOGICAL GROUNDWATER MONITORING AND LABORATORY CONSULTATION

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Project Summary

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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

Introduction: 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. Over the past ten years, enzyme-based methodologies which simultaneously detect both total coliforms and *E. coli*, have become widely accepted as the standard for water microbiological testing. These tests are based on the detection of the enzymes Beta-D galactosidase and Beta-D glucuronidase which are uniquely associated with total coliforms and *E. coli* respectively. Enzyme based coliform and *E. coli* tests must include enhancements in order to work effectively in a variety of water matrices. For example, buffers, salts and micro-nutrients are added to enhance enzyme expression. These additives are particularly important in tests that allow enumeration, where the enzyme production from a single organism must be detected. Another important ingredient might be an antibiotic added to suppress the activity of non coliforms while leaving the coliforms unaffected. For example, *Aeromonas*, a

non-coliform, is known to produce small amounts of Beta-D galactosidase. A concentration of 1000 unsuppressed *Aeromonas* in a water sample could trigger a false positive result.

During the 1990's, the three USEPA approved enzyme based methods were thoroughly tested, characterized and subsequently became widely used for testing Wisconsin groundwaters. Recently, seven new enzyme-based products have been approved by the USEPA. While others have reported specific problems with some of the newly approved products, to date, no comprehensive studies detailing the side-by-side performance of these new tests have been published (14, 15). Since the Wisconsin State Laboratory of Hygiene serves as statewide center of expertise in water testing methodologies, preliminary work was performed in the authors' laboratory which suggested differences in the efficacy of some of newly approved products. These early findings led to the project described in this report. 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).

Procedures and Methods: The ten enzyme based tests evaluated during this project are all USEPA approved products for drinking water analysis. Although there are many similarities in the approved methodologies, many of the tests have distinctive characteristics and features. The test methods evaluated are: Colilert, Colilert-18, Colisure, mColiBlue 24, Readycult Coliforms 100, Coliscan, E*Colite, Chromocult, MI agar and Colitag. Three sampling sites were chosen to include geographically, geologically and chemically diverse groundwaters. The chemistry of each groundwater was determined using Standard Methods analyses for alkalinity, pH, hardness, conductivity and soluble iron (3). Samples were tested from two sampling events and the results are summarized in Table 1. Site I is from a high hardness source that was softened using ion exchange resin. Site I is characterized by a high pH, alkalinity and conductivity and a low amount of soluble iron and a low hardness due to softening. Site II was moderately hard water with a neutral pH, and moderate alkalinity and conductivity. Site III had a low hardness, pH, alkalinity, and conductivity.

Table 1. Chemical characteristics of the sampling sites

	Site I		**Site II**		**Site III**	
pH	8.1	8.4	7.4	7.4	6.44	6.26
Alkalinity(mg/L)	331.53	331.17	100	101.1	10.22	9.87
Hardness(mg/L)	3.36	3.97	100.2	98.15	12.5	11.11
Soluble Iron(mg/L)	0.002	0.004	0.15	0.39	0.19	0.07
Conductivity(uS/cm)	898	891	202.1	201.4	117	106.7

Each site was sampled on two occasions, once for the total coliform and *E. coli* detection and quantification experiments (Objectives 1 and 2) and again for the *Aeromonas* suppression experiments (Objective 3), for a total of six sampling visits. Each sample consisted of 60 liters of water collected in three, 20 liter Cubitainers™ and transported to the laboratory within 24 hours of collection.

Samples for use in objectives 1 and 2 were prepared by dispensing 100 milliliter samples from the Cubitainers™.mixed with magnetic stir bars. For each of the three (3) sites, five different total coliform organisms (5) were spiked at two different concentrations (2) and tested in triplicate (3)

for each of the eleven methods (11) for a total of 990 samples. 100 mL samples were also prepared from each of the three sites (3) for use in objective 3. Each sample was spiked with two strains of *Aeromonas* (2) prepared at six different ten fold dilutions (6) tested in triplicate (3) for each of the eleven methods (11) for a total 1188 objective 3 samples.

The total coliform (*E. coli, Klebsiella spp., Enterobacter spp., Citrobacter spp., and Serratia spp*.) and *Aeromonas* spp. organisms used for this study were isolated from actual drinking water samples analyzed at the Wisconsin State Laboratory of Hygiene. These single passage environmental organisms were isolated, identified and frozen at -80^{o} C prior to use in this study. The cultures were prepared for spiking the day before the spiking procedure by thawing at room temperature, inoculating onto nutrient agar slants and incubating for 24 hours at 35^{o} C. The next day, the isolates were harvested from the slant into a 99 milliliter blank of phosphate buffered dilution water (4). Serial dilutions were then performed to create bacterial suspensions containing low levels(1-10) and high levels(50-100) organisms per mL which were subsequently added as one mL inoculums to the 100 ml samples for use in the experiments(3, 4). These spike levels were chosen to represent microbe levels actually seen in water supply contamination events. The actual spike concentrations of the prepared dilutions were determined using a Heterotrophic Plate Count test (3). Since the Colisure and E*colite test systems allow the test to be read out at either 24 or 48 hours, (28 or 48 for E*Colite) results were determined and recorded at both times.

For the total coliform and *E. coli* detection portion of the experiment, triplicate pairs of individual water samples were spiked with one mL aliquots of the spike material from each strain of bacteria at levels that resulted in 1-10 and 50-100 bacteria in the 100 mL test vials. For the *Aeromonas spp*. suppression procedure, two strains of *Aeromonas spp*. were obtained through the same culture protocol as described above. One-hundred mL samples spiked with $10^{1,}10^{2}$,10^{3},10^{4},10^{5} and 10^{6} of each *Aeromonas spp.* strain were prepared. This spiking protocol was repeated on three separate days for each of the three sampling sites.
As the samples were spiked, a heterotrophic plate count was performed within 30 minutes for each of the prepared suspensions to determine the actual spike concentration. An unspiked "blank" of the sample water was tested using each enzyme-based product and Ampicillin-Dextrin agar with vancomycin(4) to determine any background total coliform or *Aeromonas spp*. in the unspiked water which could influence the results of the spiked samples. Each lot of the enzyme-based products used for this project was tested using a positive control, a negative control and a sterility check.
After completion of the spiking procedure, the samples were processed using each of the test methods, following the protocols provided by the manufacturers. (5, 6, 7, 8, 9, 10, 11, 12, 13,).

Results and Discussion: Federal regulations regarding the occurrence of total coliforms and *E. coli* in drinking water are based on the presence or absence of the organisms rather than the numbers of organisms detected. Consequently any test method must be capable of reliably producing this presence/absence result. Tables 2, 3 and 4 simply show the ability of each of the evaluated methods to detect total coliforms and *E. coli* in a presence/absence format. In most cases, the triplicate analyses performed on each sample to increase the robustness of the data set had identical results between the replicates. There were two exceptions on the low level concentration of *Klebsiella*, where the triplicate analysis yielded two "present" results and one "absent". These results were treated as a present result in the tables. The expected result for these first objective trials was "coliform present" and, for the *E. coli* spike, "*E coli* present" for all samples. Surprisingly, this was not the case in 32 of the 330 tests. This 9.7% false negative rate is clearly a concern.

For Site 1, (Table 2.) the test methods were capable of detecting total coliforms and *E. coli* with three exceptions. Colilert-18 and E*Colite incubated for 28 hours were unable to detect *Serratia spp.* at both spike levels of <10 and 50-100 cells. In addition, the E*Colite product also failed to detect the <10 *Citrobacter spp*. spike. Table 3 shows a failure to detect *Serratia spp.* using Colisure read at 24 hours, but not at 48 hours, E*Colite read at both 28 and 48 hours and Readycult at one or sometimes both of the spike levels in site 2. Detection variability was the worst at site 3 (Table 4) For example, Colisure read at 24 and 48 hours failed to detect *Enterobacter spp.* at the <10 concentration and *Citrobacter spp.* at both <10 and 50-100 cells. mColiBlue24 was incapable of detecting *E. coli*, *Klebsiella spp*. and *Citrobacter spp*. at both spike levels and *Enterobacter spp.* at <10 cells. Readycult and E*Colite read at 28 hours did not detect *Serratia spp.* at both spike levels. A summary of the failure rates for each test is presented in Table 5 below.

Colilert, Coliscan, MI agar, Chromocult, and Colitag performed as expected and were all capable of detecting the presence of total coliforms and *E. coli* in all samples tested. The most alarming of these results is the inability of m-ColiBlue 24 to detect *E. coli* at the CWI site. The failures of the various methods appear to be both organism and sample matrix dependant.

Table 2. Presence/Absence Data Results for Site I

	Site I P=Present A=Absent									
	Citrobacter spp.		***Enterobacter spp.***		***E. coli***		***Klebsiella spp.***		***Serratia spp.***	
Product	**<10**	**50-100**	**<10**	**50-100**	**<10**	**50-100**	**<10**	**50-100**	**<10**	**50-100**
Colilert	P	P	P	P	P	P	P	P	P	P
Colilert-18	P	P	P	P	P	P	**A**	P	P	P
Colisure-24	P	P	P	P	P	P	P	P	**A**	**A**
Colisure-48	P	P	P	P	P	P	P	P	P	P
Coliscan w/CF	P	P	P	P	P	P	P	P	P	P
Coliscan	P	P	P	P	P	P	P	P	P	P
MI Agar	P	P	P	P	P	P	P	P	P	P
mColiBlue 24	P	P	P	P	P	P	P	P	P	P
Chromocult	P	P	P	P	P	P	P	P	P	P
Readycult	P	P	P	P	P	P	P	P	**A**	**A**
E*Colite-28	**A**	P	P	P	P	P	P	P	**A**	**A**
E*Colite-48	P	P	P	P	P	P	P	P	P	P
Colitag	P	P	P	P	P	P	P	P	P	P

Table 3. Presence/Absence Data Results for Site 2

	Site 2				P=Present		A=Absent			
	Citrobacter spp.		***Enterobacter spp.***		***E. coli***		***Klebsiella spp.***		***Serratia spp.***	
Product	**<10**	**50-100**	**<10**	**50-100**	**<10**	**50-100**	**<10**	**50-100**	**<10**	**50-100**
Colilert	P	P	P	P	P	P	P	P	P	P
Colilert-18	P	P	P	P	P	P	P	P	P	P
Colisure-24	P	P	P	P	P	P	P	P	**A**	P
Colisure-48	P	P	P	P	P	P	P	P	P	P
Coliscan w/CF	P	P	P	P	P	P	P	P	P	P
Coliscan	P	P	P	P	P	P	P	P	P	P
MI Agar	P	P	P	P	P	P	P	P	P	P
mColiBlue 24	P	P	P	P	P	P	P	P	P	P
Chromocult	P	P	P	P	P	P	P	P	P	P
Readycult	P	P	P	P	P	P	P	P	**A**	**A**
E*Colite-28	P	P	P	P	P	P	P	P	**A**	**A**
E*Colite-48	P	P	P	P	P	P	P	P	**A**	**A**
Colitag	P	P	P	P	P	P	P	P	P	P

Table 4. Presence/Absence Data Results for Site 3

	Site 3				P=Present		A=Absent			
	Citrobacter spp.		***Enterobacter spp.***		***E. coli***		***Klebsiella spp.***		***Serratia spp.***	
Product	**<10**	**50-100**	**<10**	**50-100**	**<10**	**50-100**	**<10**	**50-100**	**<10**	**50-100**
Colilert	P	P	P	P	P	P	P	P	P	P
Colilert-18	P	P	P	P	P	P	P	P	P	P
Colisure-24	**A**	**A**	**A**	P	P	P	P	P	P	P
Colisure-48	**A**	**A**	**A**	P	P	P	P	P	P	P
Coliscan w/CF	P	P	P	P	P	P	P	P	P	P
Coliscan	P	P	P	P	P	P	P	P	P	P
MI Agar	P	P	P	P	P	P	P	P	P	P
mColiBlue 24	**A**	**A**	**A**	P	**A**	**A**	**A**	**A**	P	P
Chromocult	P	P	P	P	P	P	P	P	P	P
Readycult	P	P	P	P	P	P	P	P	**A**	**A**
E*Colite-28	P	P	P	P	P	P	P	P	**A**	**A**

E*Colite-48	P	P	P	P	P	P	P	P	P	P
Colitag	P	P	P	P	P	P	P	P	P	P

Table 5. A summary of the failure rates for each presence/absence test

USEPA Approved Product	Failure Rate
Colilert	0%
Colilert-18	3.3%
Colisure-24	20%
Colisure-48	10%
Coliscan w/CF	0%
Coliscan	0%
MI Agar	0%
mColiBlue 24	23.3%
Chromocult	0%
Readycult	20%
E*Colite-28	20%
E*Colite-48	6.7%
Colitag	0%

In addition to the ability to simply detect the presence/absence of total coliforms and *E. coli*, some of the USEPA approved test systems also have the ability to enumerate organisms. In the enumeration portion of the experiments, analyses were once again done in triplicate. The triplicate test results were arithmetically averaged and compared to the heterotrophic plate count results as a recovery percentage. Preliminary observations of the data showed that there was an apparent difference in enumeration capabilities of the tests based on the sample matrix. Consequently, Figures 1 and 2 depict the percent recovery of each of the spike organisms for each enumeration capable method stratified by sample site (matrix). Figure 1 shows the results low level spike (1-10 organisms) and Figure 2 displays the high level spike (50-100 organisms). The stratified graphs allow facile comparisons of recoveries for all of the methods and each of the organisms across all three sample types.

The most obvious observation is the inability of Colisure and mColiBlue 24 to significantly recover any level of coliforms spiked into the CWI water. This result was so striking that it was suspected that testing error might have been involved. It was decided to rerun this portion of the analysis in order to rule out this possibility. The retest resulted in verification of the initial results.

It also becomes apparent that individual test methods vary in their ability to recover specific coliform organisms. For example Colilert 18 does a poor job in recovery of *Klebsiella*. Another example is that Colisure, when read at 24 hours does a poor job recovering *Serratia*. The other valuable information gleaned from these graphs is the obvious effect of sample matrix on the ability of individual test systems to recover the spiked organisms.

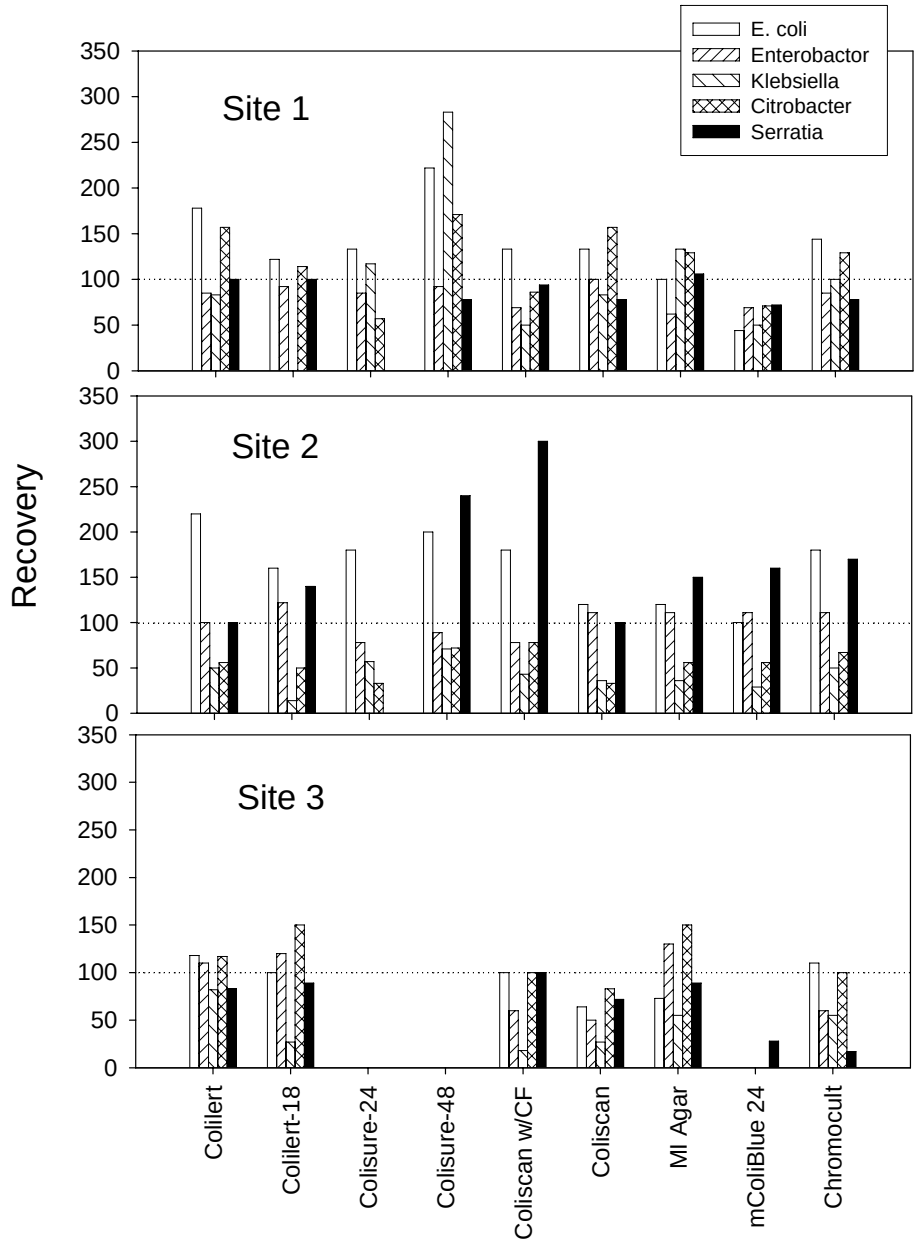

Figure 1. Percent recovery data for samples spiked with 1-10 bacteria.

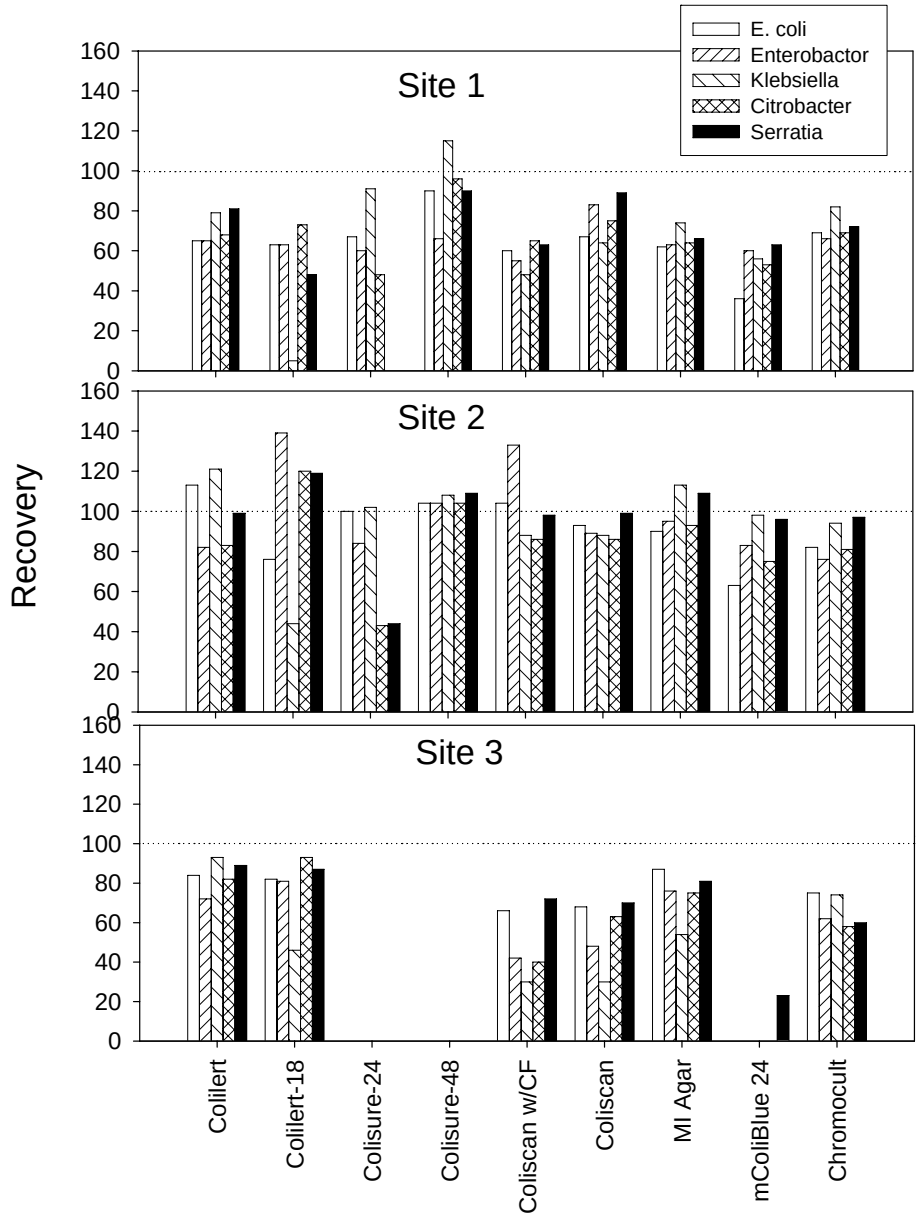

Figure 2. Percent recovery data for samples spiked with 50-100 bacteria.

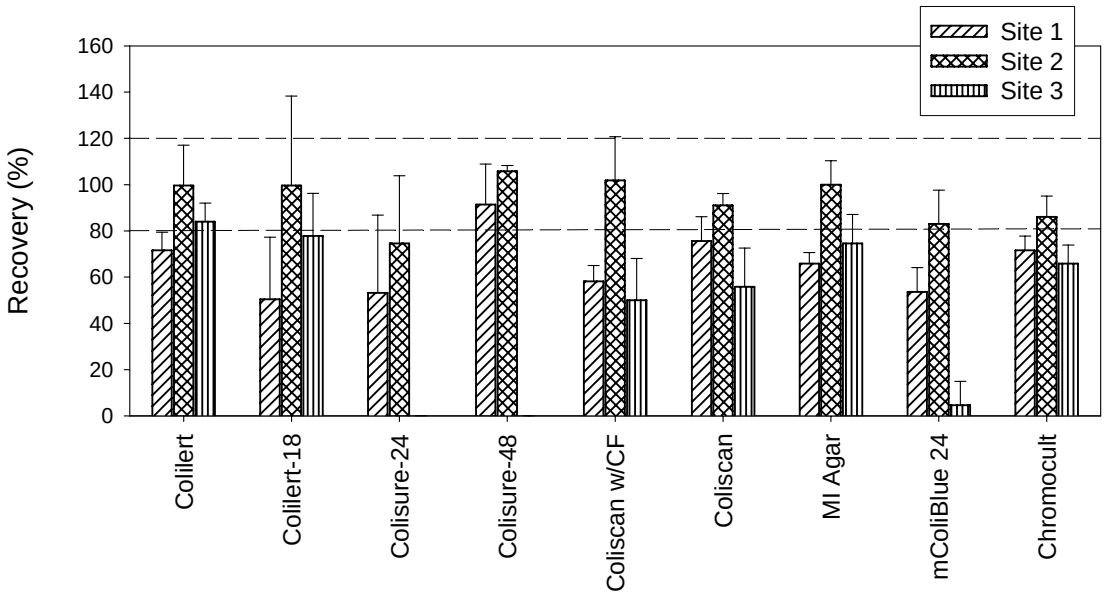

Figure 3. Average percent recovery plots for all samples spiked with 50-100 organisms including error bars representing one standard deviation from the mean.

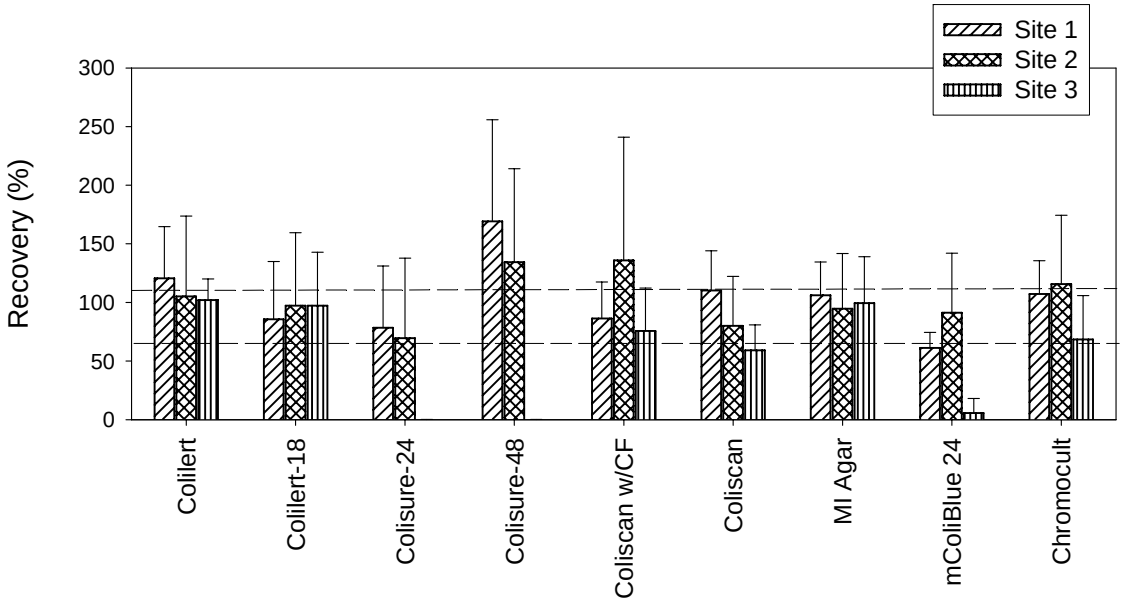

Figure 4. Average percent recovery plots for all samples spiked with 1-10 organisms including error bars representing one standard deviation from the mean.

With each site representing different water quality characteristics, the figures (figures 3 and 4) for each test spiked with 50-100(high) and 1-10(low) organisms show significant differences among the methods ability to recover the spiked organisms at a 100% level. The figures represent the mean recovery for all total coliform organisms for each test at each site. The error bars represent

one standard deviation from the mean. The useful information to take away from these figures is the fact that tests with error bars that overlap 100% are have expected recoveries that are not statistically different from 100% but tests with error bars that cover a broad range are expected to have results that have wide variations. Clearly, all tests had higher variability for the low spikes, which is a result of the larger uncertainty in the spike determination for the low level spikes. Since the Safe Drinking Water Act states that only one organism detected is considered an unsafe sample and a follow-up sample is necessary, this information could be useful for decisions regarding which enzymatic test to use for determining the efficiency of water treatment.

Perhaps the most important aspect of a product's performance lies in its ability to perform on real world samples where large numbers of non target organisms can interfere with the test results. Objective 3 of this project was aimed at testing this aspect of product performance. The expected result is that a product will suppress the growth and galactosidase production of the non-coliform organism even when the non-coliform organism level is in the 10^5 range. The data from the objective three experiments are presented in Tables 6, 7 and 8, once again stratified by sampling site. The data indicates that there was a wide diversity in the ability of the various products to suppress *Aeromonas spp.* For site 1, Colilert, Colilert-18, Readycult, E*Colite at 28 hours of incubation, Colitag, and Coliscan with and without Cefsulodin all displayed suppression capability at all spike levels. The other products were unable to suppress *Aeromonas spp.* at various contamination levels. The Chromocult product could not suppress *Aeromonas spp.* even at the minimum spike level.

Site 1

Organism	***Aeromonas spp*. strain #1**						***Aeromonas spp*. strain #2**					
Spike Amount	10^0	10^1	10^2	10^3	10^4	10^5	10^0	10^1	10^2	10^3	10^4	10^5
Product												
Colilert	-	-	-	-	-	-	-	-	-	-	-	-
Colilert-18	-	-	-	-	-	-	-	-	-	-	-	-
Colisure-24	-	-	-	-	+	-	-	-	-	-	-	-
Colisure-48	-	-	-	-	+	-	-	-	-	-	-	-
Readycult	-	-	-	-	-	-	-	-	-	-	-	-
E*Colite-28	-	-	-	-	-	-	-	-	-	+	-	+
E*Colite-48	-	-	-	-	+	+	+	+	+	+	+	+
Colitag	-	-	-	-	-	-	-	-	-	-	-	-
Coliscan w/CF	-	-	-	-	-	-	-	-	-	-	-	-
Coliscan	-	-	-	-	-	-	-	-	-	-	-	-
MI Agar	-	-	-	-	-	-	-	-	+	+	+	+
mColiBlue 24	-	-	-	-	-	-	-	-	-	+	+	+
Chromocult	+	+	+	+	+	+	+	+	+	+	+	+

Table 6. Product ability to suppress two different strains of *Aeromonas spp*. at the Southern Wisconsin Site

Site 2

Organism	***Aeromonas spp*. strain #1**						***Aeromonas spp*. strain #2**					
Spike Amount	10^0	10^0	10^1	10^2	10^3	10^5	10^0	10^1	10^2	10^3	10^4	10^5
Product												
Colilert	-	-	-	-	-	-	-	-	-	-	-	+
Colilert-18	-	-	-	-	-	-	-	-	-	-	-	-
Colisure-24	-	-	-	-	-	-	-	-	-	-	-	-
Colisure-48	+	+	-	-	-	+	-	+	-	-	-	-
Readycult	-	-	-	-	-	-	-	-	-	-	-	-
E*Colite-28	-	-	-	-	-	-	-	+	+	+	+	+
E*Colite-48	+	+	+	+	+	+	+	+	+	+	+	+
Colitag	-	-	-	-	-	-	-	-	-	+	+	-
Coliscan w/CF	-	-	-	-	-	-	-	+	+	+	+	+
Coliscan	-	-	-	-	+	+	-	-	-	+	+	+
MI Agar	-	-	-	-	-	-	+	+	+	+	+	+
mColiBlue 24	-	-	-	-	-	-	+	+	+	+	+	+
Chromocult	+	+	+	+	+	+	+	+	+	+	+	+

Table 7. Product ability to suppress two different strains of *Aeromonas spp*. at the Northern Wisconsin Site

Site 3

Organism	***Aeromonas spp*. strain #1**						***Aeromonas spp*. strain #2**					
Spike Amount	10^0	10^1	10^2	10^3	10^4	10^5	10^0	10^1	10^2	10^3	10^4	10^5
Product												
Colilert	-	-	-	-	-	-	-	-	-	-	-	-
Colilert-18	-	-	-	-	-	-	-	-	-	-	-	-
Colisure-24	-	-	-	-	-	-	-	-	-	-	-	-
Colisure-48	-	-	-	-	-	-	-	-	-	-	-	-
Readycult	-	-	-	-	-	-	-	-	-	-	-	-
E*Colite-28	-	-	-	-	-	-	-	-	-	-	+	+
E*Colite-48	+	+	+	+	+	+	+	+	+	+	+	+
Colitag	-	-	-	-	-	-	-	-	-	-	-	-
Coliscan w/CF	+	+	+	+	+	+	-	+	+	+	+	+
Coliscan	+	+	+	+	+	+	+	+	+	+	+	+
MI Agar	-	-	-	-	+	+	-	-	-	-	-	-
mColiBlue 24	-	-	-	-	-	-	-	-	-	-	-	-
Chromocult	+	+	+	+	+	+	+	+	+	+	+	+

Table 8. Product ability to suppress two different strains of *Aeromonas spp*. at the Central Wisconsin Site

Discussion

Enzyme based methodologies have become widely accepted as the industry standard for water microbiological testing. The USEPA has approved ten of these methods for use in testing drinking water, irregardless of the fact there is a paucity of side-by-side comparison data available that labs can use in choosing a product for purchase. The data produced in this study suggests that there are significant differences between the ten USEPA approved methods both in the ability to detect 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. 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 CWI site 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. The background heterotrophic plate count (HPC) for the site 3 groundwater was 418 cfu/ml, whereas the background counts for the site 1 and site 2 were 16 cfu/ml and 3 cfu/ml respectively. This increased level of heterotrophic bacteria may have influenced the ability of mColiBlue24 and Colisure products to provide accurate results whereas the other products were less sensitive to background bacteria.

The presence of high levels of *Aeromonas spp.* in water samples, which may have a low level of galactosidase production, can lead to false positive results if the organisms are not adequately suppressed by the media additives. In this study, major differences between products and their ability to suppress *Aeromonas spp*. were observed. Tables 6, 7 and 8 indicated differences in product abilities to suppress *Aeromonas spp.* between sites and between *Aeromonas spp.* strains. There was no apparent pattern to each product's inability to suppress *Aeromonas spp.* The chemical characteristics that defined the sampling sites appeared to have no effect on the amount of *Aeromonas spp*. that the methods could or could not suppress. With the exception of Colilert-18 and Readycult, all methods at some point in this study were unable to suppress *Aeromonas spp*. Variability exists for enzyme-based products to suppress different strains of *Aeromonas spp.* For example, the strain 1 *Aeromonas* seeded in site 1 and site 2 site water was completely suppressed in MI agar however, MI agar failed to suppress *Aeromonas spp.* strain number 2. Conversely, MI agar failed to suppress *Aeromonas spp.* strain number 1 and completely suppressed strain number 2 seeded into the site 3 source. Product inconsistencies were also observed. In some instances, a product would be unable to suppress *Aeromonas spp*. at a lower spike level yet completely suppress *Aeromonas spp.* seeded at a greater spike level. This finding remains unexplained and will require further investigation.

Further research with enzyme-based methods is needed to increase the amount of data to better understand the implications of these results. Additional investigation regarding how chemical characteristics and amount of background heterotrophic bacteria may affect detection of total coliforms and *E. coli* when using enzyme-based technology is also needed. Future research will focus more on which methods are best capable of accurately detecting low levels of chlorine-

stressed total coliform and *E. coli* as well as properly suppressing *Aeromonas spp*. as well as other non-coliform bacteria that may interfere with proper operation of the enzyme-based product. The study does point out the need for carefully side-by-side evaluations of any product in the actual environment it will be used prior to use in any testing where the results will be used for making public health decisions.

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