A comparison of USEPA approved enzyme-based total coliform / E.coli tests for groundwater monitoring

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Introduction

- Protection of groundwater is a top priority for environmental water regulators as well as private well owners
- Recent epidemiological studies show that gastrointestinal illness due to ingestion of drinking water is occurring at significant levels in the U.S. and Canada.
- US Center for Disease Control study indicated that 70% of outbreaks occurring in the U.S. were associated with well water sources

What is needed?

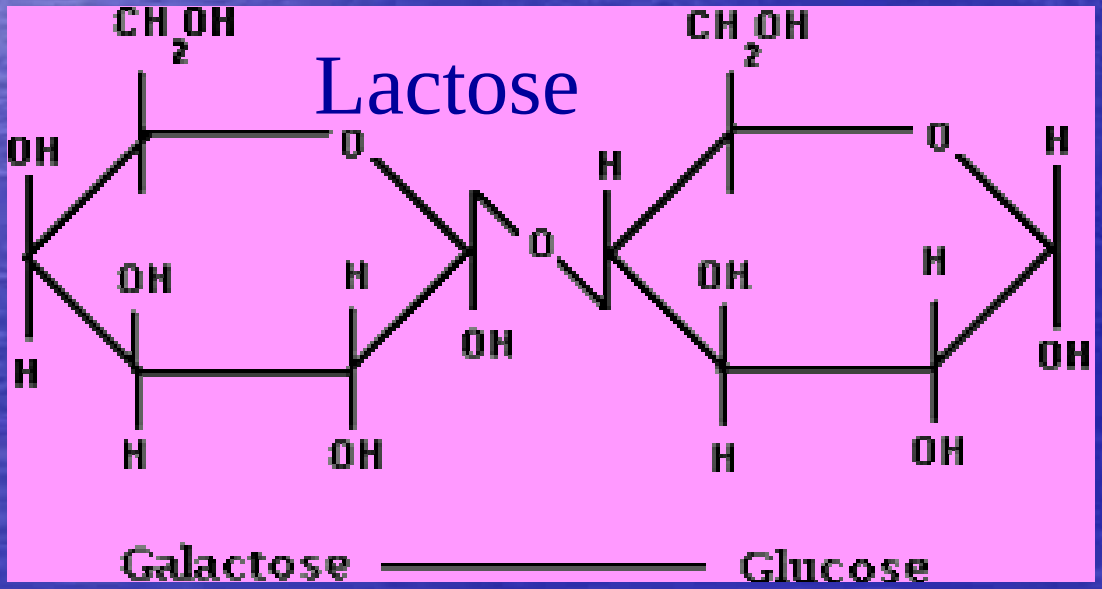
- Sensitive, reliable and affordable laboratory methods to detect microbial contamination
- Over the past 100 years methods for detection of total coliform and E. coli have evolved
- Enzyme-based methods have become industry standard

What are enzyme-based methods?

- Total coliform and E. coli produce unique enzymes
- Total coliform produce beta-D Galactosidase
- *E. coli* produce beta-D Glucuronidase
- These tests are based on the ability of each enzyme to hydrolyze lactose

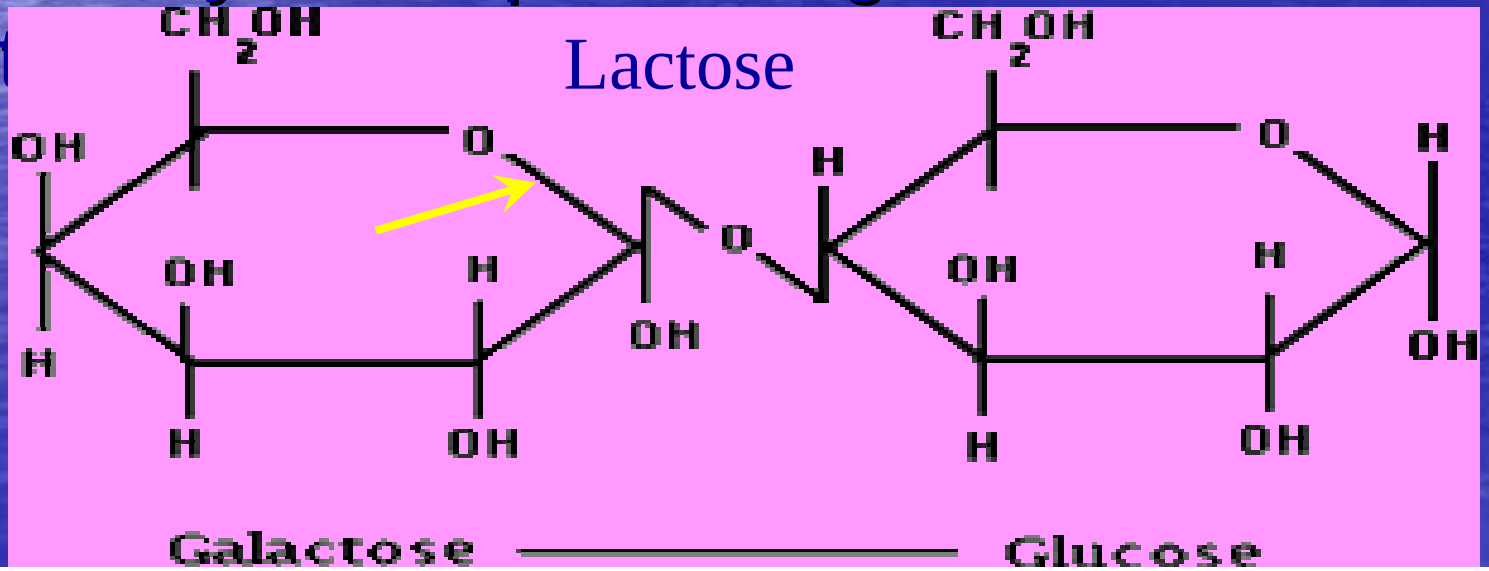
Unique Total Coliform Characteristics When Cultured

- Lactose(milk sugar) fermentation
  - Lactose hydrolyzed to form lactic acid + hydrogen gas + carbon dioxide gas.



Unique Total Coliform Enzyme

- Many enzymes involved in lactose hydrolosis
- Galactosidase is only produced by total coliforms
- This enzyme helps the organism break down lact

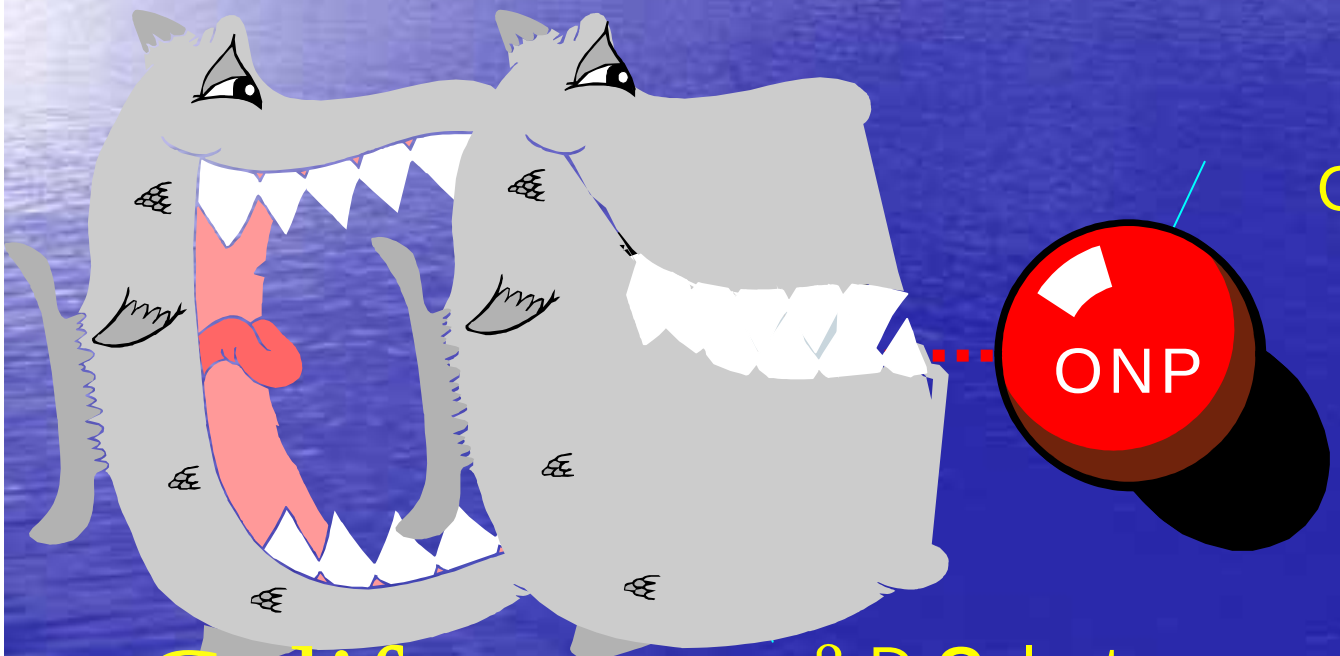


ONPG Positive Reaction

Colilert

ONPG=

Ortho-NitroPhenyl + β-D-Galactopyranoside



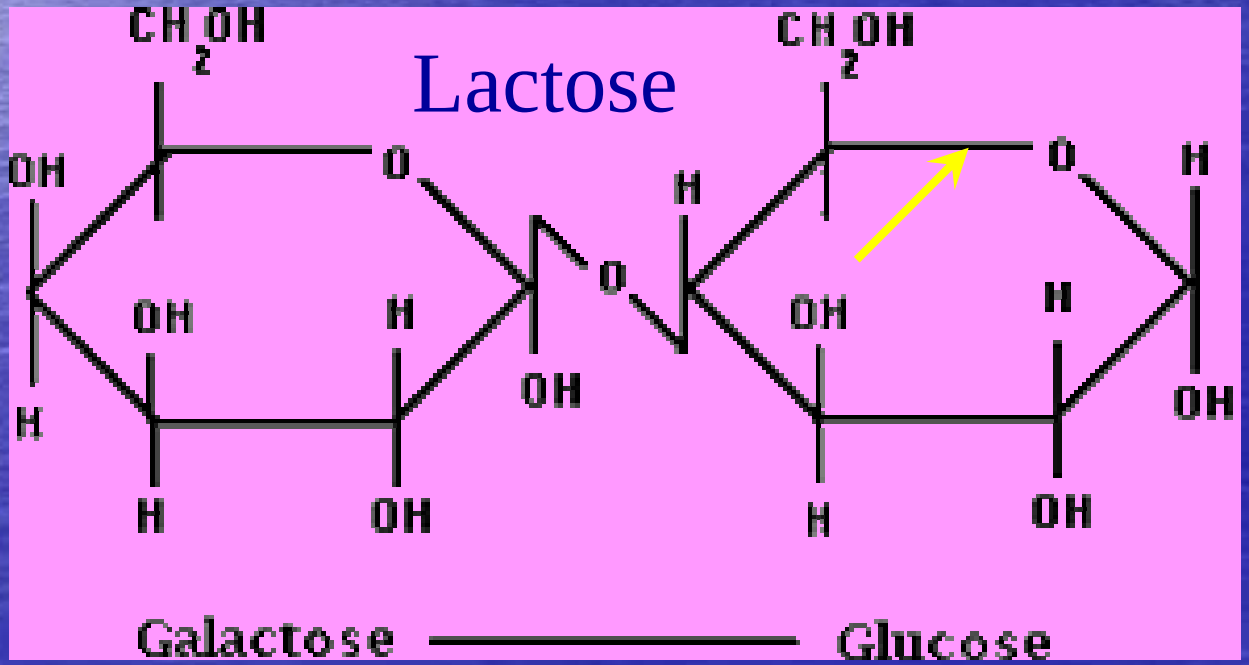
Ortho-NitroPhenyl

Coliform

β-D-**G**alactopyranoside
(sugar molecule)

Unique *E. coli* Enzymatic Characteristic

- Produces the enzyme Glucuronidase which speeds up lactose hydrolysis through glucuronide hydrolysis

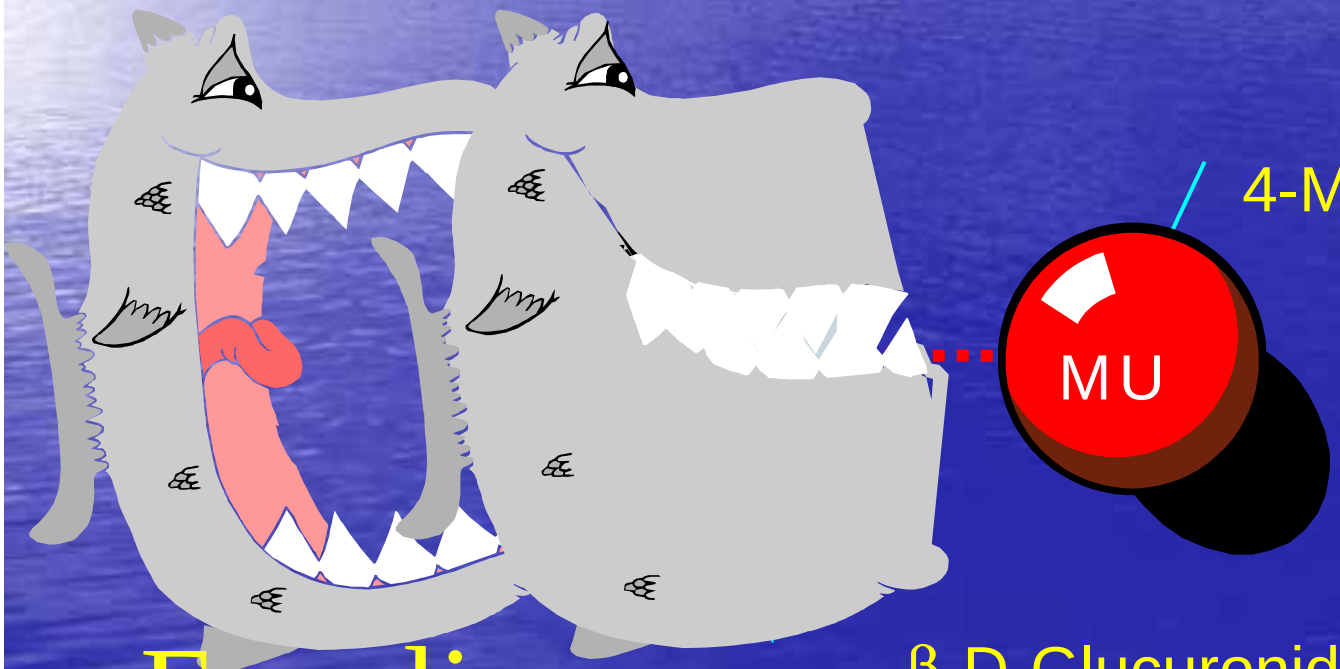


MUG Positive Reaction

Colilert

MUG=

4-Methyl-Umbelliferyl + β-D-Glucuronide



E. coli

4-MethylUmbelliferyl

MU

β-D-Glucuronide
(sugar molecule)

Result of Lactose Hydrolysis by Enzymes

- Enzyme-based tests result in a chromogen and fluorogen being released.
- Some enzyme-based tests only result in different color chromogens being released to represent total coliform and/or *E. coli* presence.
- Chromogens and fluorogens released are triggered by galactosidase and glucuronidase hydrolysing the lactose substrates.

EPA Approved Enzyme-Based Tests

- Prior to 2001, three enzyme-based tests were approved by U.S. EPA
- All three of these methods were thoroughly tested for use for groundwater testing.
- More recently the U.S. EPA has approved many other enzyme-based tests for use.

Approved enzyme-based total coliform and *E. coli* tests

- Prior to 2001 – Colilert(IDEXX), Colilert-18(IDEXX) and Colisure(IDEXX)
- Seven recently approved tests – Coliscan(Micrology Laboratories), mColi Blue 24 (Hach/Millipore), Readycult Coliforms 100(EMD Chemicals Inc.), Chromocult(EMD Chemicals), E*Colite(Charm Sciences Inc.), MI Agar(S&S Biosciences), Colitag(CPI International).

Recently Approved Enzyme-Based Tests

- Limited amount of data available regarding the newly approved tests
- No published studies detailing a side-by-side performance of all approved tests
- No studies regarding their performance to the best of our knowledge are currently underway

Objective

- Determine the capabilities of each product to detect total coliform and E. coli in three chemically diverse Wisconsin groundwaters
- Determine the ability of each product to accurately quantify the number of total coliforms and E. coli in WI groundwaters
- Determine the ability of each test to of suppress *Aeromonas spp*.
- Determine the unit cost for each test system

Sampling Sites

- Three sampling sites representing geographically and chemically diverse groundwaters
- Site I – high pH, alkalinity and conductivity, low soluble iron and hardness due to softening
- Site II – moderate hardness with neutral pH,moderate alkalinity and conductivity
- Site III – low hardness, pH, alkalinity and conductivity

Chemical characterization of sites

- Chemical analysis was conducted each time a collection was made from each site
- Analysis for alkalinity, pH, hardness, conductivity and soluble iron content was conducted

Project Design

- Each site was sampled twice
  - Once for the total coliform detection objectives
  - Once for *Aeromonas spp.* suppression experiments
- Samples were dispensed and spiked with five total coliforms separately at two different levels of contamination. Each spiked in triplicate for the total coliform objectives.
- Similarly, the *Aeromonas spp.* objectives consisted of dispensing 100mL samples and spiking with six different contaminatioin levels of two different strains of *Aeromonas spp.* in triplicate.

Chemical characteristics of the groundwater sampling sites

	Southern Wisconsin		Northern Wisconsin		Central Wisconsin	
pH	8.1	8.4	7.4	7.4	6.44	6.26
Alkalinity(mg/L)	332	331	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

Results

- Some methods were unable to detect certain species of total coliform in Wisconsin groundwaters.
- Major differences were seen in the ability of each test to quantify amounts of total coliform and *E. coli*.
- Vast dissimilarities were seen between methods and strains to suppress *Aeromonas spp*.

Presence/Absence Data Results – Site I

	Citrobacter		Enterobacter		E. coli		Klebsiella		Serratia	
Spike Level	<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

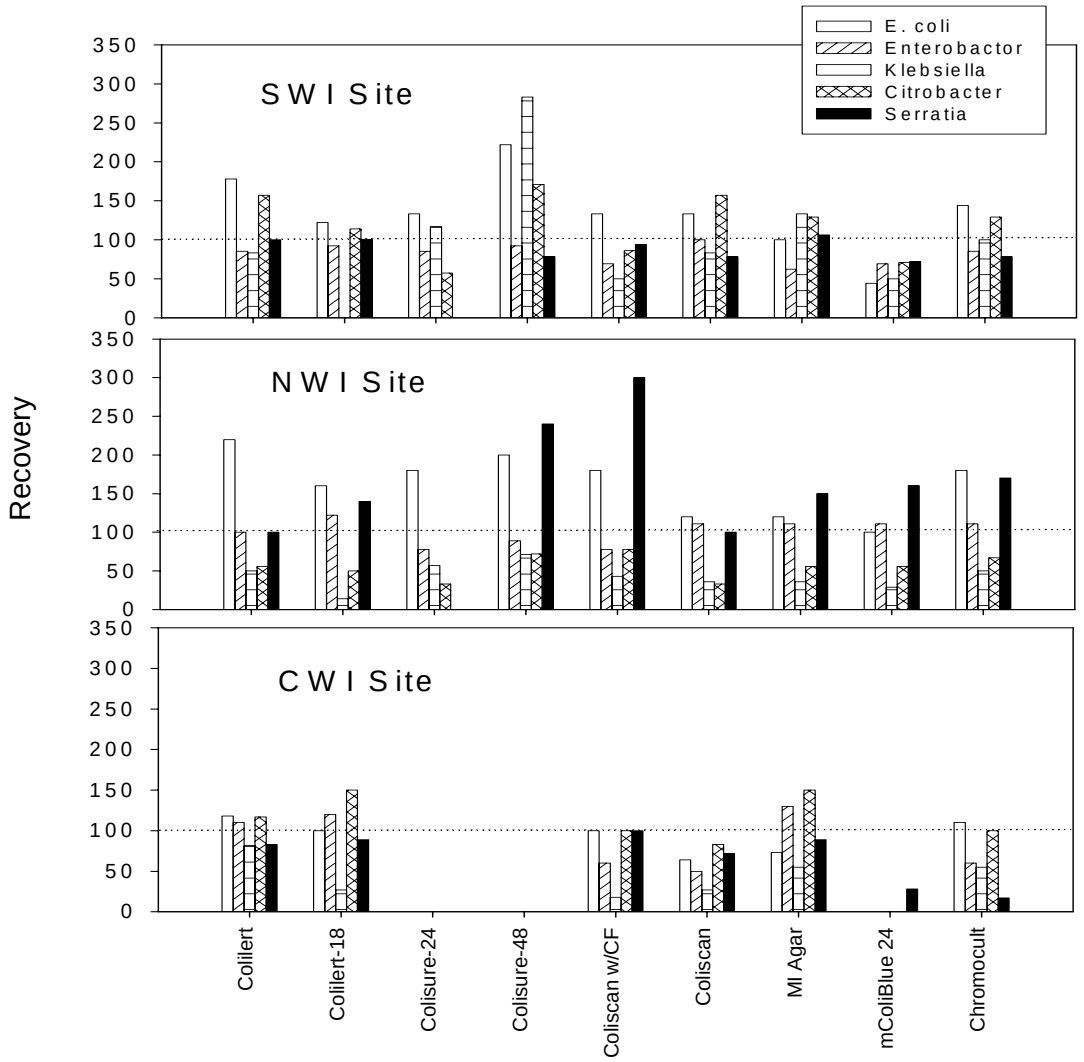
Presence/Absence Data Results – Site II

	Citrobacter		Enterobacter		E. coli		Klebsiella		Serratia	
Spike Level	<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

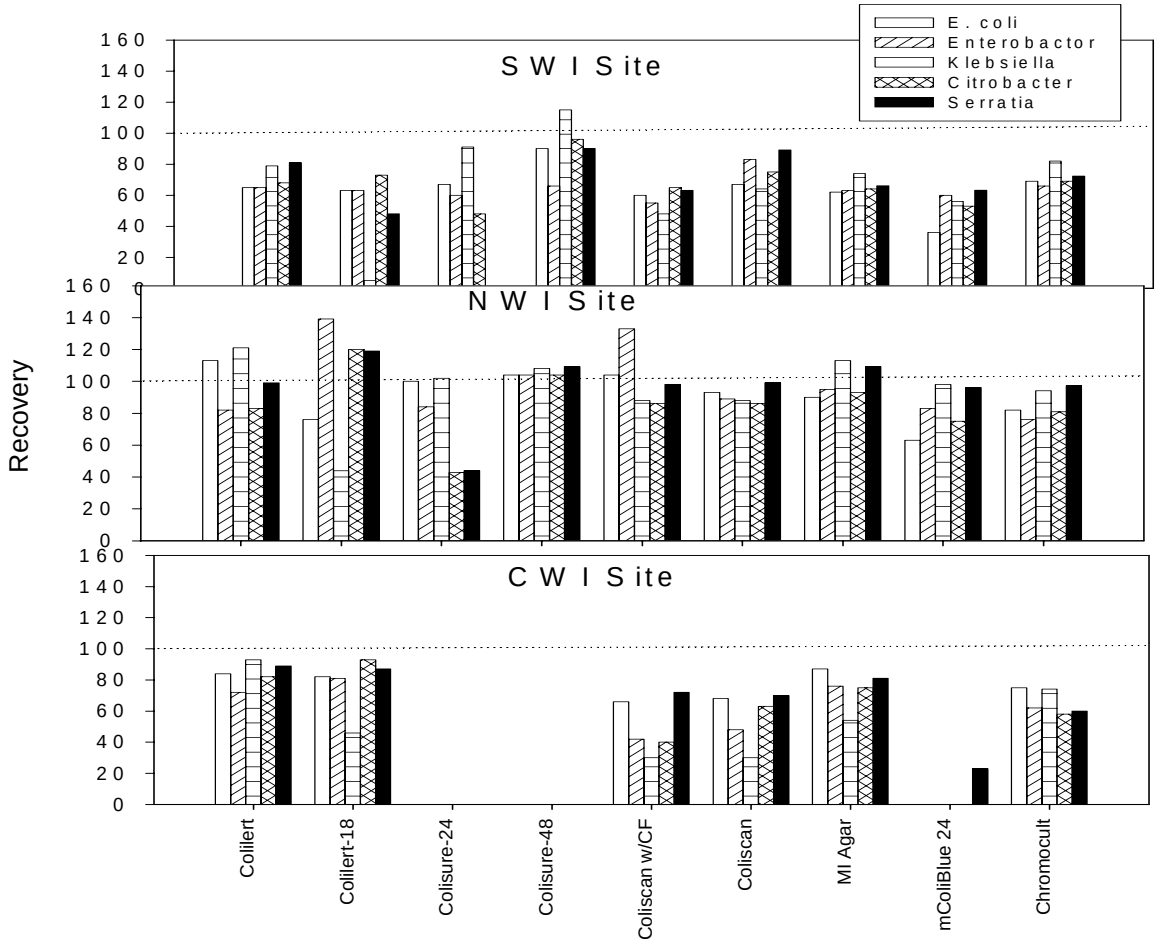
Presence/Absence Data Results – Site III

	Citrobacter		Enterobacter		E. coli		Klebsiella		Serratia	
Spike Level	<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	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

Samples Spiked with <10 Organisms



Samples Spiked with 50-100 Organisms



Product Ability to Suppress Aeromonas spp. – Site I

	Aeromonas spp			Strain #1				*Aeromonas spp*			Strain #2		
Spike Level	10^1	10^2	10^3	10^4	10^5	10^6		10^1	10^2	10^3	10^4	10^5	10^6
Colilert	-	-	-	-	-	-		-	-	-	-	-	-
Colilert-18	-	-	-	-	-	-		-	-	-	-	-	-
Colisure-24	-	-	-	-	+	-		-	-	-	-	-	-
Colisure-48	-	-	-	-	+	-		-	-	-	-	-	-
Coliscan w/CF	-	-	-	-	-	-		-	-	-	-	-	-
Coliscan	-	-	-	-	-	-		-	-	-	-	-	-
MI Agar	-	-	-	-	-	-		-	-	+	+	+	+
mColiBlue 24	-	-	-	-	-	-		-	-	-	+	+	+
Chromocult	+	+	+	+	+	+		+	+	+	+	+	+
Readycult	-	-	-	-	-	-		-	-	-	-	-	-
E*Colite-28	-	-	-	-	-	-		-	-	-	+	-	-
E*Colite-48	-	-	-	-	+	+		+	+	+	+	+	+
Colitag	-	-	-	-	-	-		-	-	-	-	-	-

Product ability to suppress Aeromonas spp. – Site II

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

Product ability to suppress Aeromonas spp. – Site III

	Aeromonas spp			Strain #1				*Aeromonas spp*			Strain #2		
Spike Level	10^1	10^2	10^3	10^4	10^5	10^6		10^1	10^2	10^3	10^4	10^5	10^6
Colilert	-	-	-	-	-	-		-	-	-	-	-	-
Colilert-18	-	-	-	-	-	-		-	-	-	-	-	-
Colisure-24	-	-	-	-	-	-		-	-	-	-	-	-
Colisure-48	-	-	-	-	-	-		-	-	-	-	-	-
Coliscan w/CF	+	+	+	+	+	+		-	+	+	+	+	+
Coliscan	+	+	+	+	+	+		+	+	+	+	+	+
MI Agar	-	-	-	-	+	+		-	-	-	-	-	-
mColiBlue 24	-	-	-	-	-	-		-	-	-	-	-	-
Chromocult	+	+	+	+	+	+		+	+	+	+	+	+
Readycult	-	-	-	-	-	-		-	-	-	-	-	-
E*Colite-28	-	-	-	-	-	-		-	-	-	-	+	+
E*Colite-48	+	+	+	+	+	+		+	+	+	+	+	+
Colitag	-	-	-	-	-	-		-	-	-	-	-	-

Discussion

- Some methods were unable to detect certain species of total coliform in Wisconsin groundwaters.
- Major differences were seen in the ability of each test to quantify amounts of total coliform and *E. coli*.
- Vast dissimilarities were seen between methods and strains to suppress *Aeromonas spp*.

Simple Detection of total coliform and *E. coli*

- Some methods from the CWI site exhibited failure to simply detect total coliform and *E. coli*
- Figures 1 and 2 graphically illustrate the overall lower % recoveries of total coliform and *E. coli* from the CWI site
- One possible explanation is the water quality characteristics

Presence/Absence Data Results – Site III

	Citrobacter		Enterobacter		E. coli		Klebsiella		Serratia	
Spike Level	<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	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

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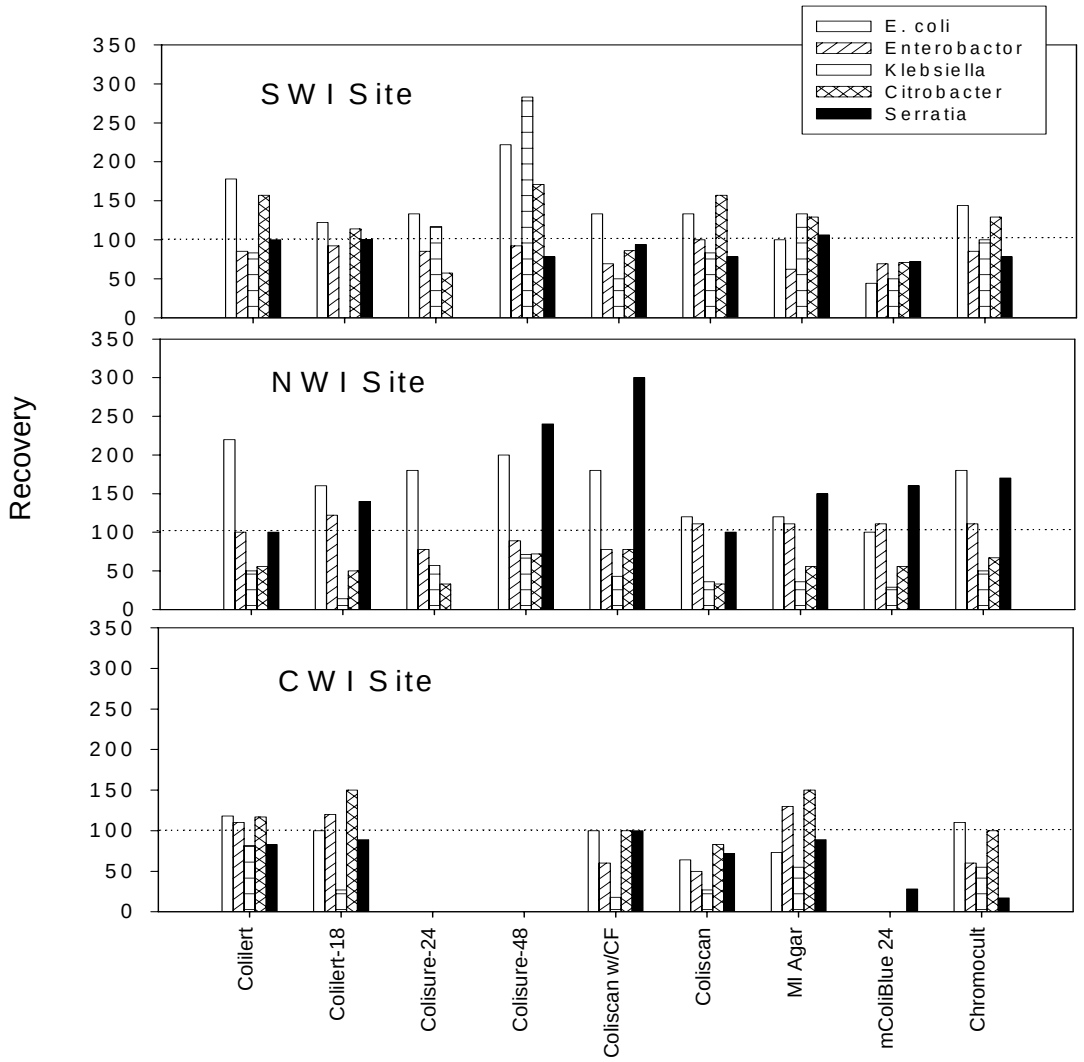
Samples Spiked with <10 Organisms



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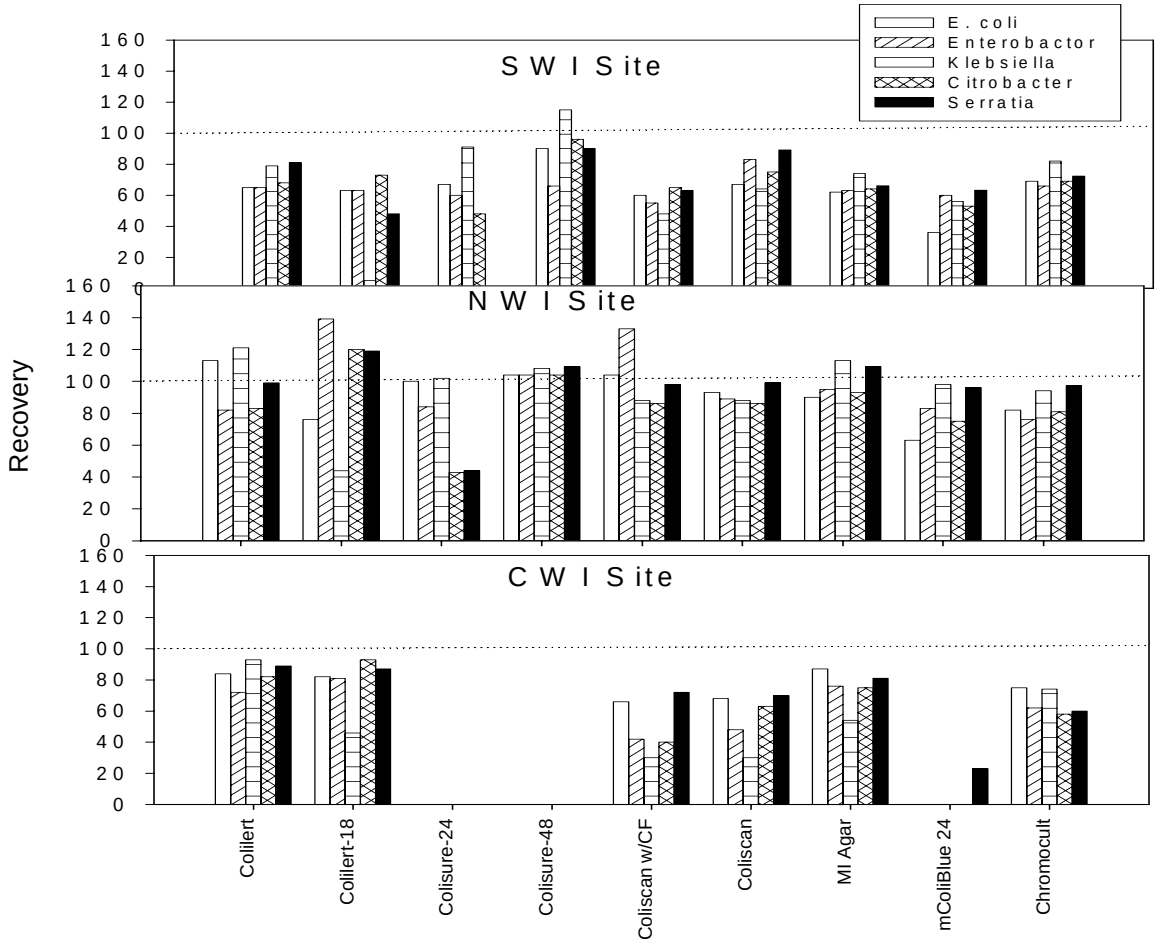

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Chemical characteristics of the groundwater sampling sites

	Site I		Site II		Site III	
pH	8.1	8.4	7.4	7.4	6.44	6.26
Alkalinity(mg/L)	332	331	100	101.1	10.22	9.87
Hardness(mg/L)	3.36	3.97	100.2	98.15	12.5	11.11
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Conductivity(uS/cm)	898	891	202.1	201.4	117	106.7

Possible Water Quality Interferences of Enzyme-Based Tests

- High levels of background heterotrophic bacteria interference
- Low pH
  - Media may require a greater buffering capacity
  - May not provide enough acid-neutralizing capacity to provide accurate results
- Low alkalinity

Analysis of *Aeromonas spp*. Suppression Objective

- Differences observed in products ability to supress *Aeromonas spp.*
- No obvious pattern to each products inability to suppress *Aeromonas spp*.
- Variability between strains of *Aeromonas spp*.
- Product inconsistencies observed

Product ability to suppress Aeromonas spp. – Site II

	Aeromonas spp			Strain #1				*Aeromonas spp*			Strain #2		
Spike Level	1	2	26	283	6093	347033		5	28	757	6393	41180	307383
Colilert	-	-	-	-	-	-		-	-	-	-	-	+
Colilert-18	-	-	-	-	-	-		-	-	-	-	-	-
Colisure-24	-	-	-	-	-	-		-	-	-	-	-	-
Colisure-48	+	+	-	-	-	+		-	-	-	-	-	-
Coliscan w/CF	-	-	-	-	-	-		-	+	+	+	+	+
Coliscan	-	-	-	-	+	+		-	-	-	+	+	+
MI Agar	-	-	-	-	-	-		+	+	+	+	+	+
mColiBlue 24	-	-	-	-	-	-		+	+	+	+	+	+
Chromocult	+	+	+	+	+	+		+	+	+	+	+	+
Readycult	-	-	-	-	-	-		-	-	-	-	-	-
E*Colite-28	-	-	-	-	-	-		-	+	+	+	+	+
E*Colite-48	+	+	+	+	+	+		+	+	+	+	+	+
Colitag	-	-	-	-	-	-		-	-	-	+	+	-

Analysis of *Aeromonas spp*. Suppression Objective(continued)

- The chemical characteristics appeared to have no effect on each products ability or inability to suppress *Aeromonas spp*.

- With the exception of Colilert-18 and Readycult Coliforms 100 all methods at some point were unable to completely suppress *Aeromonas spp*.

Summary

- Enzyme-based methodologies have become widely accepted as the industry standard for water microbiological testing
- USEPA has approved 10 different methods for total coliform and *E. coli* testing
- Data produced by this study has shown that there are major differences among all ten USEPA approved methods in the ability to detect and quantify total coliforms and *E. coli* as well as suppress *Aeromonas spp*.

Future Research Goals

- Further research intent is to generate similar data using organisms cultured from a treated public water system.
- Looking further at the effects of water quality parameters, such as pH and alkalinity, regarding enzyme-based tests.
- Spike samples with bacterial suspensions prepared by flow cytometry for increased accuracy.

Acknowledgements

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Wisconsin State Laboratory of Hygiene

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