



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

A background image showing water splashing and bubbling in a glass, with a blue-tinted overlay.

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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 clearly show that gastrointestinal disease due to ingestion of drinking water is occurring at significant levels in the United States and Canada.
- The United States Centers for Disease Control indicated that 70% of outbreaks occurring in the U.S. were associated with well water sources

What is needed?

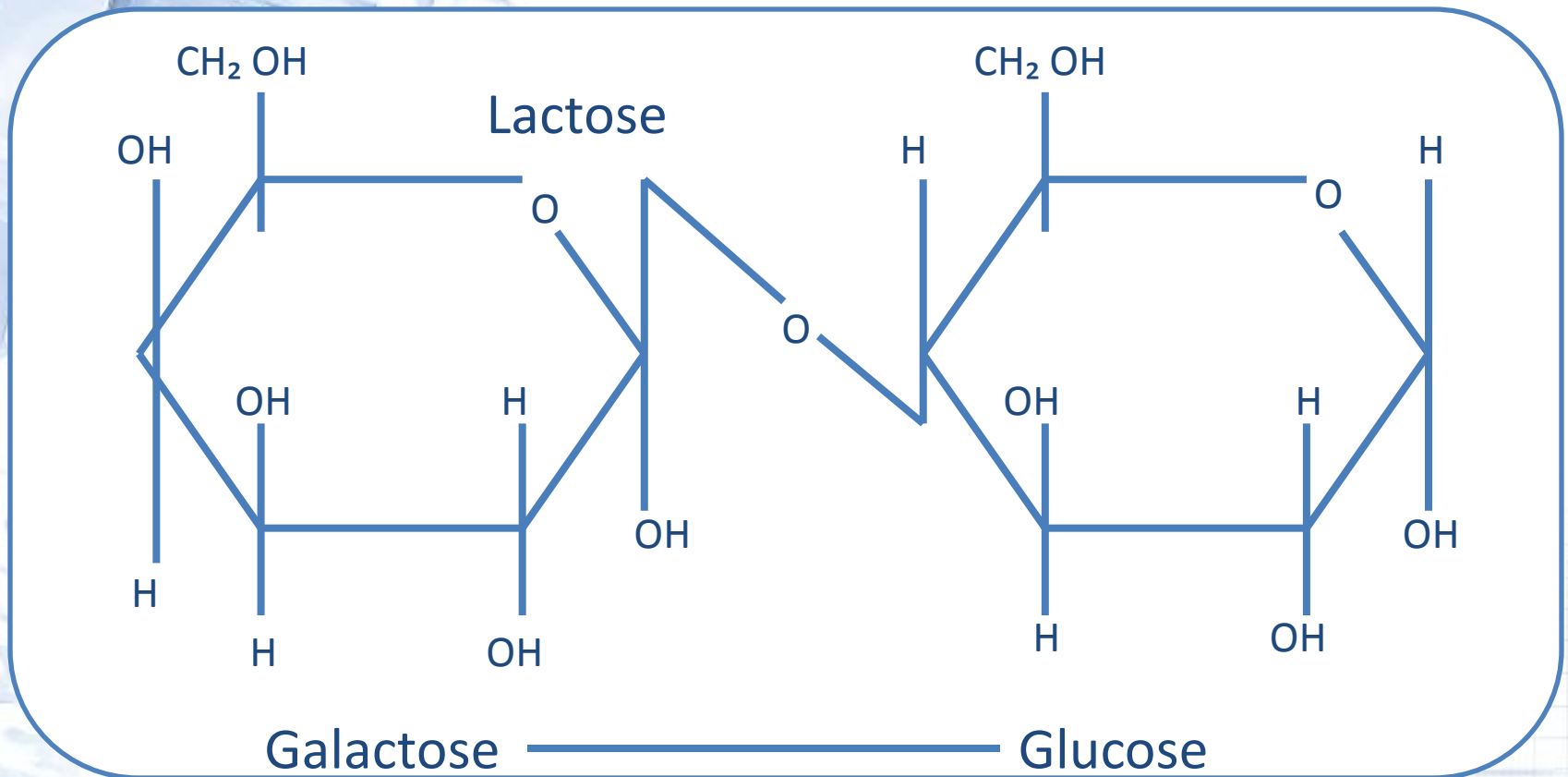
- Sensitive, reliable and affordable laboratory methods to detect microbial contamination.
- Over the past 100 years methods for detection of total coliform and E. coli have evolved.
- Enzyme-based methods have become industry standard.

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

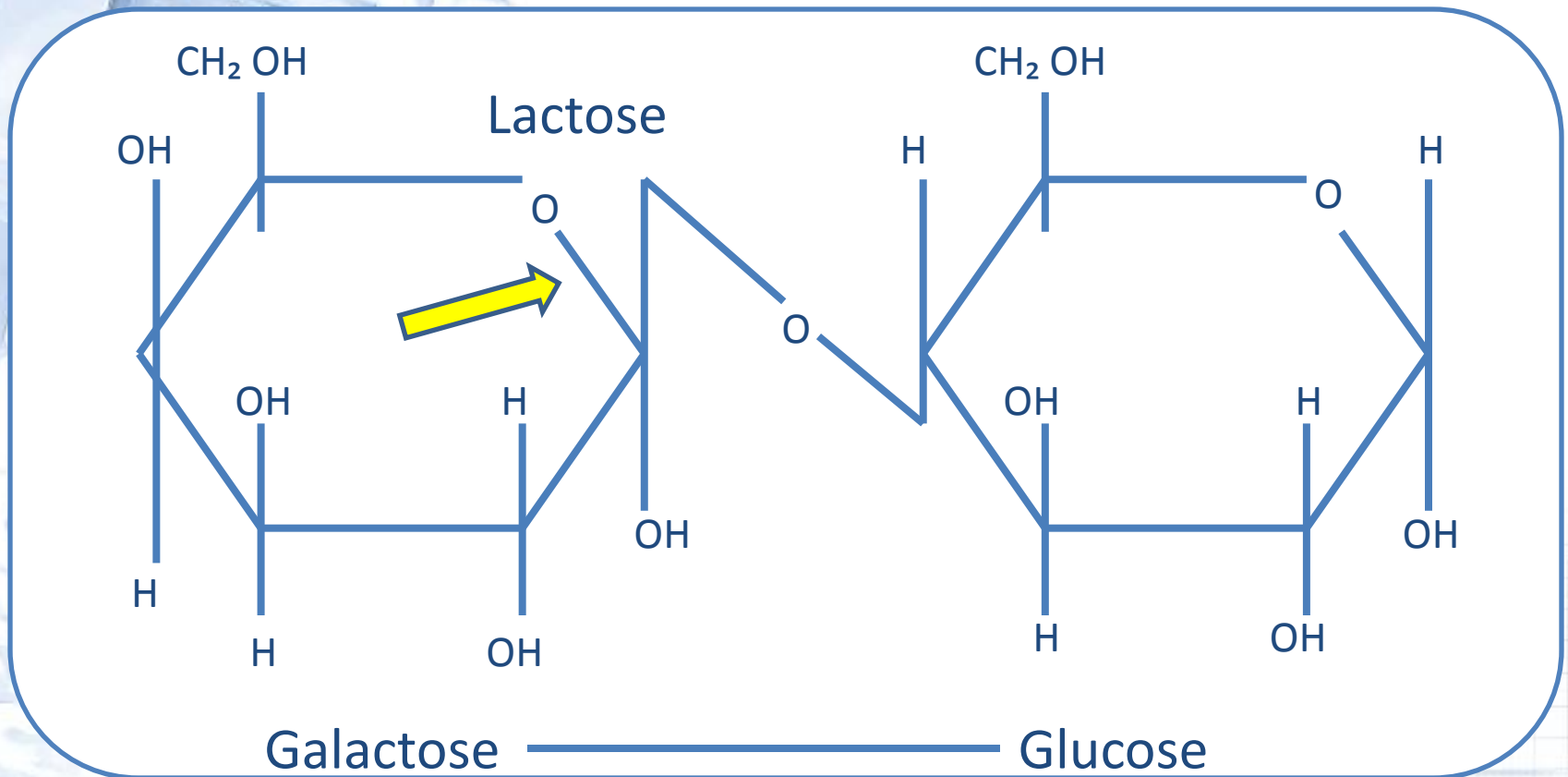
Unique Total Coliform characteristics when cultured

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



Unique Total Coliform Enzyme

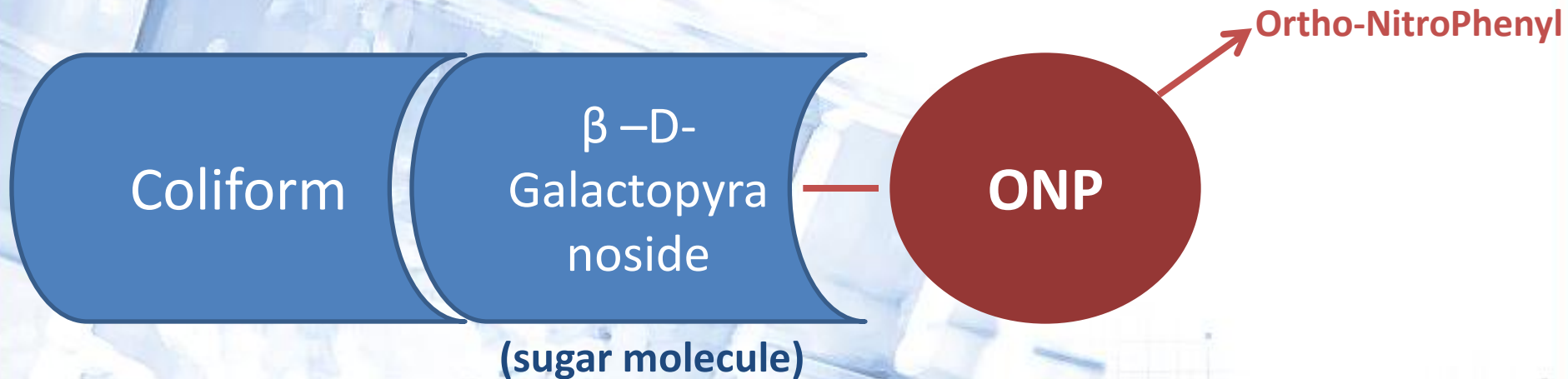
- Many enzymes involved in lactose hydrolysis
- Galactosidase is only produced by total coliforms
- This enzyme helps the organism break down lactose



ONPG Positive Reaction

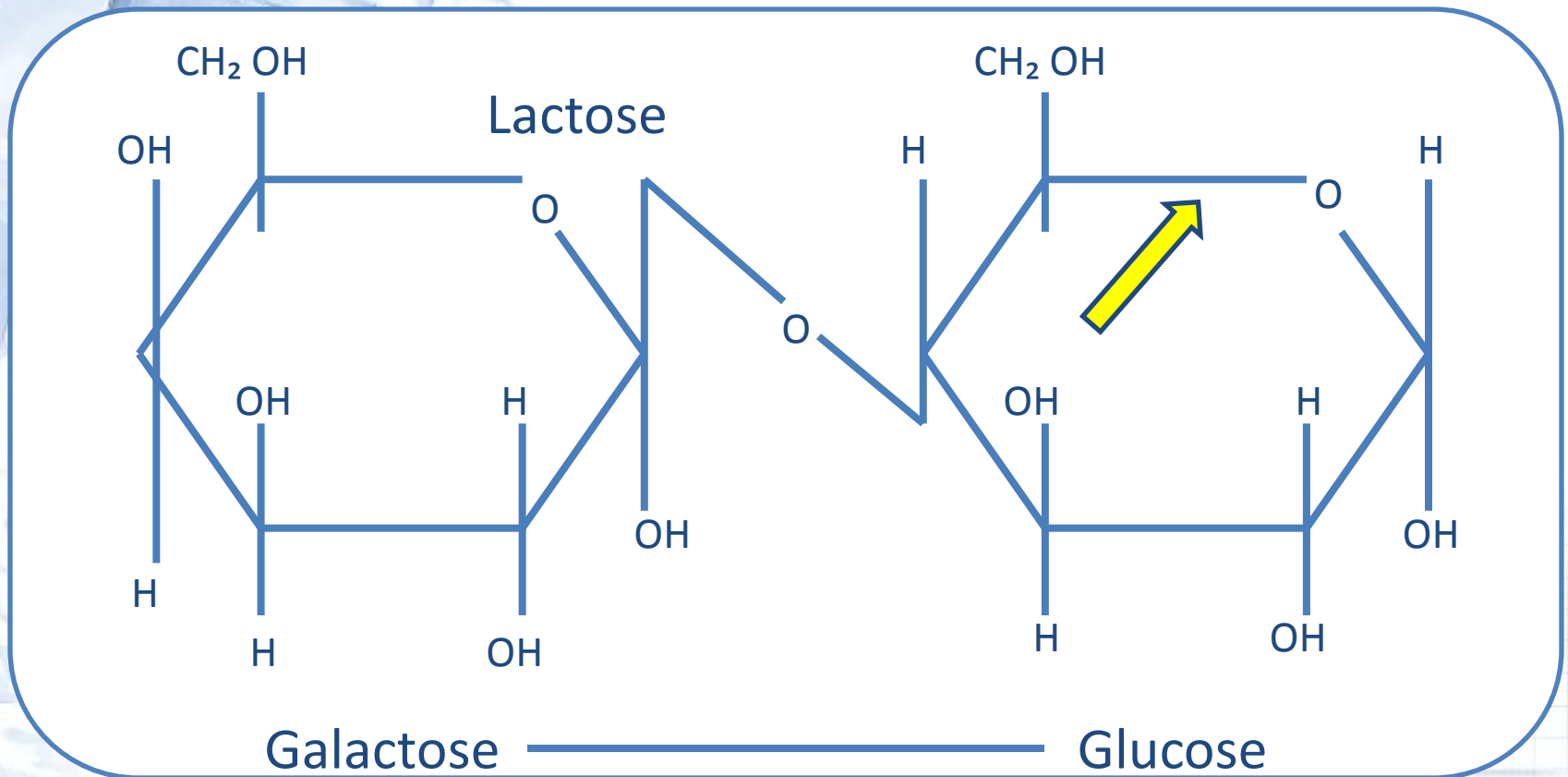
Colilert

ONPG = **O**rtho-**N**itro**P**henyl + β - D - **G**alactopyranoside



Unique *E. coli* Enzymatic Characteristic

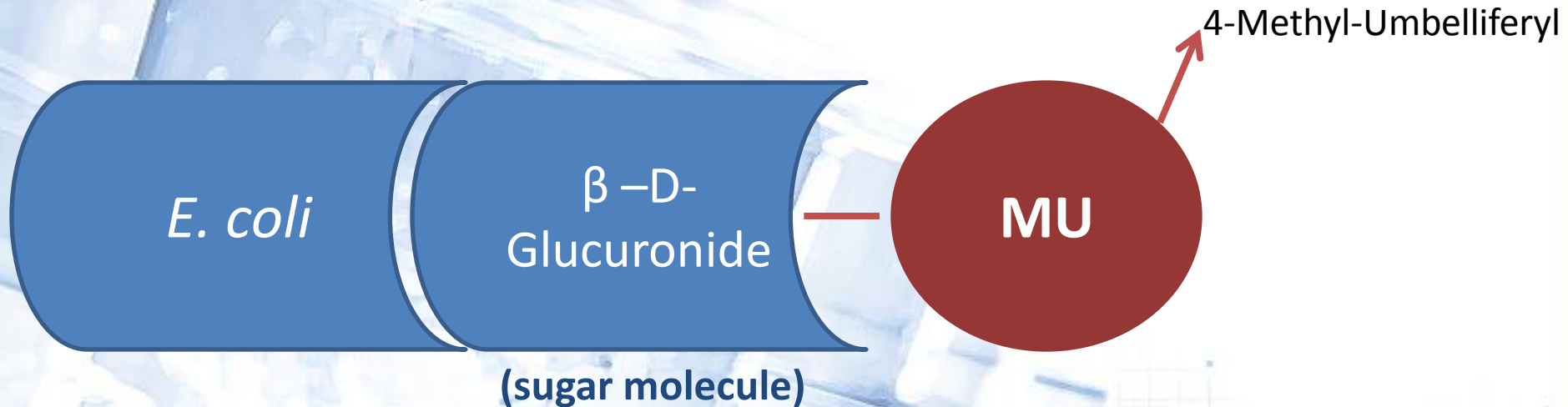
- Produces the enzyme Glucuronidase which speeds up lactose hydrolysis through glucuronide hydrolysis



MUG Positive Reaction

Colilert

MUG = 4-**M**ethyl-**U**mbelliferyl + β - D - **G**lucuronide



Result of Lactose Hydrolysis by Enzymes

- Enzyme-based 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 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 (Mycrology 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 suppress *Aeromonas spp.*
- Determine the unit cost for each 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* ssp. 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* ssp. Objectives consisted of dispensing 100mL samples and spiking with six different strains of *Aeromonas* ssp. in triplicate.

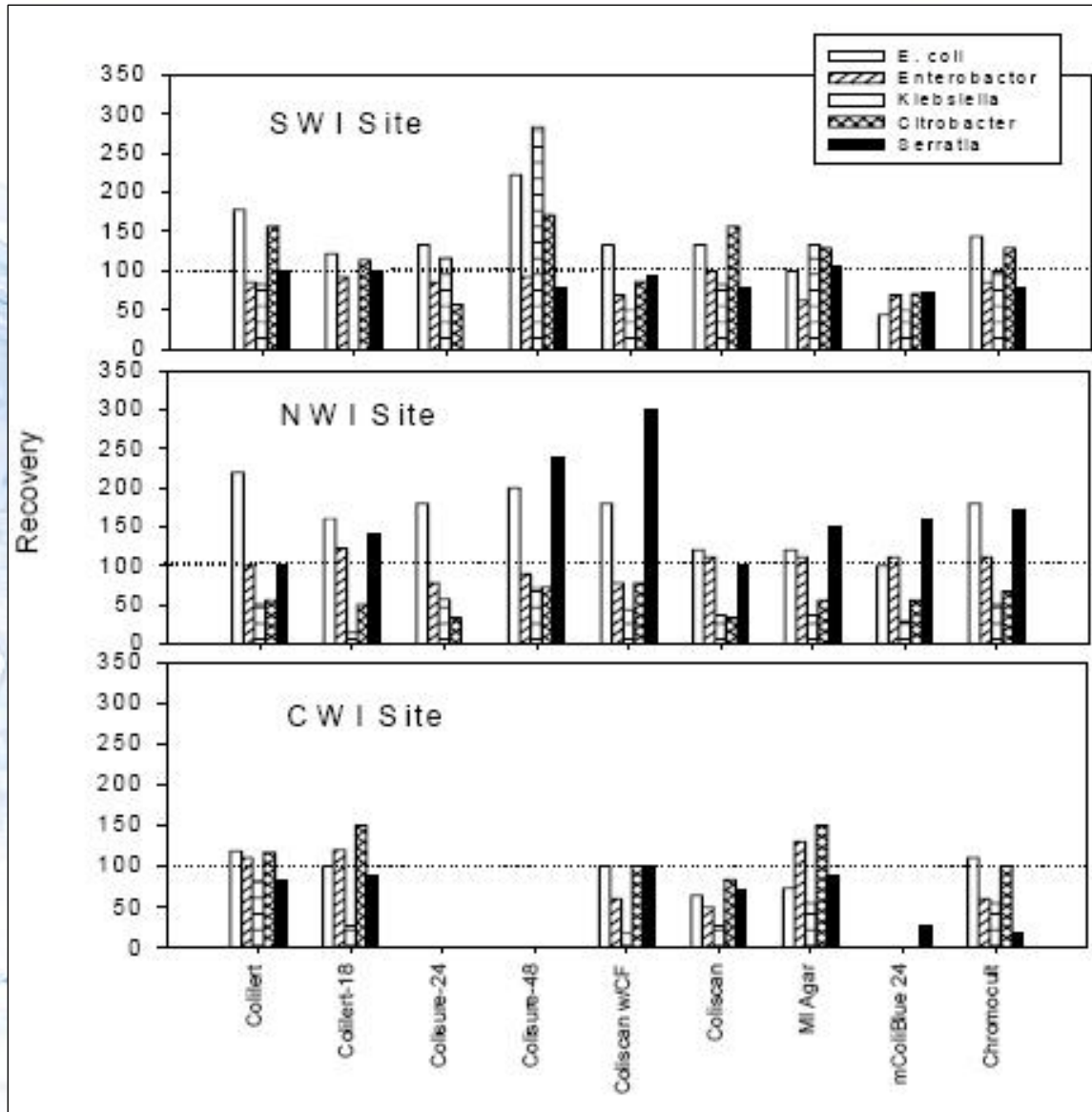
Chemical characteristics of the groundwater sampling sites

	Southern Winsconsin		Northen Winsconsin		Central Winsconsin	
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	202.4	117	106.7

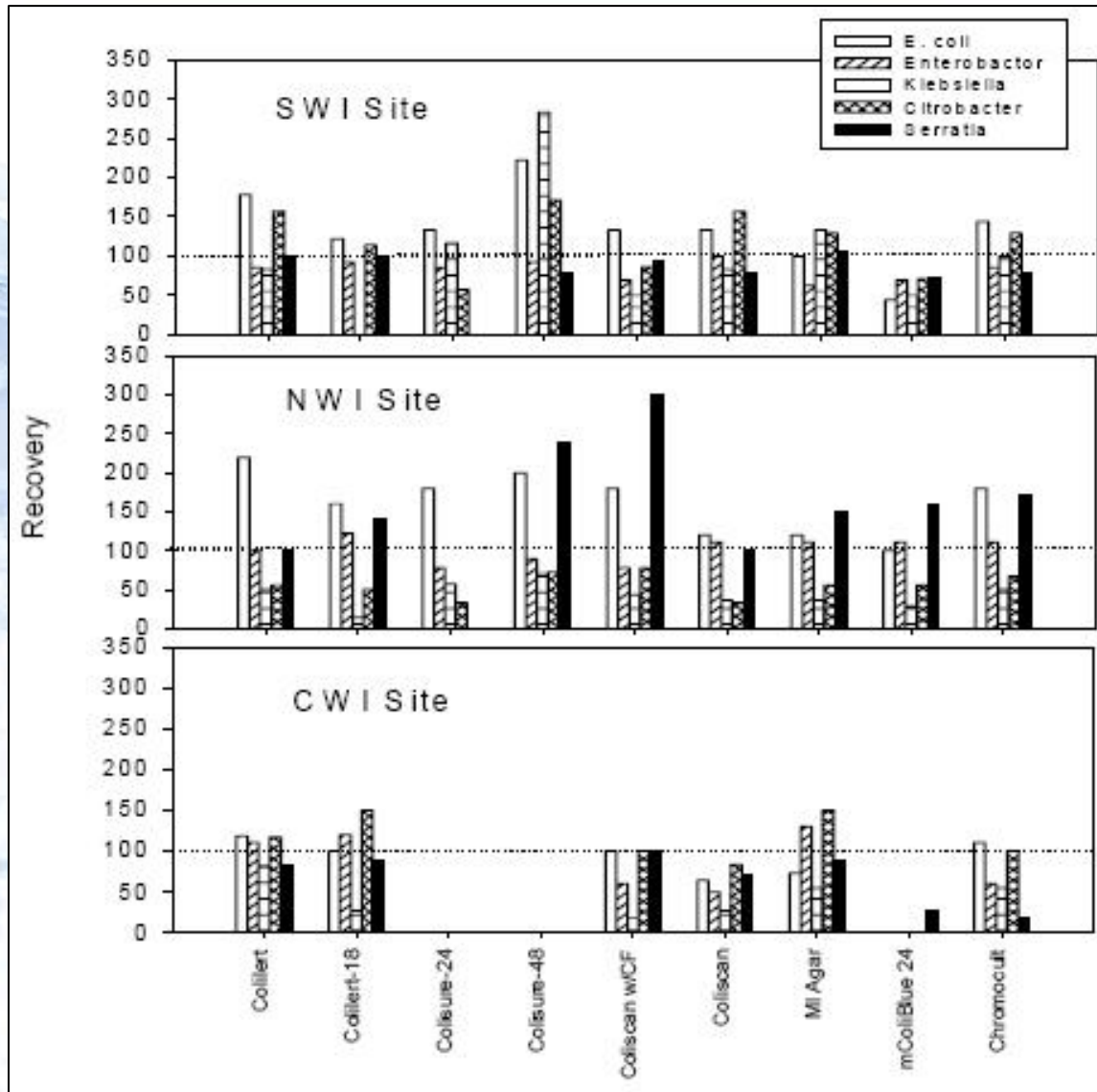
Results

- 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 ssp.*

Samples Spiked with <10 Organisms



Samples Spiked with 50-100 Organisms



Product Ability to Suppress *Aeromonas* spp. – Site II

	Aeromonas spp.			Strain #1			Aeromonas spp.			Strain #2		
	10 ⁰	10 ¹	10 ²	10 ³	10 ⁴	10 ⁵	10 ⁰	10 ¹	10 ²	10 ³	10 ⁴	10 ⁵
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	-	-	-	-	-	-	-	-	-	+	+	-

Discussion

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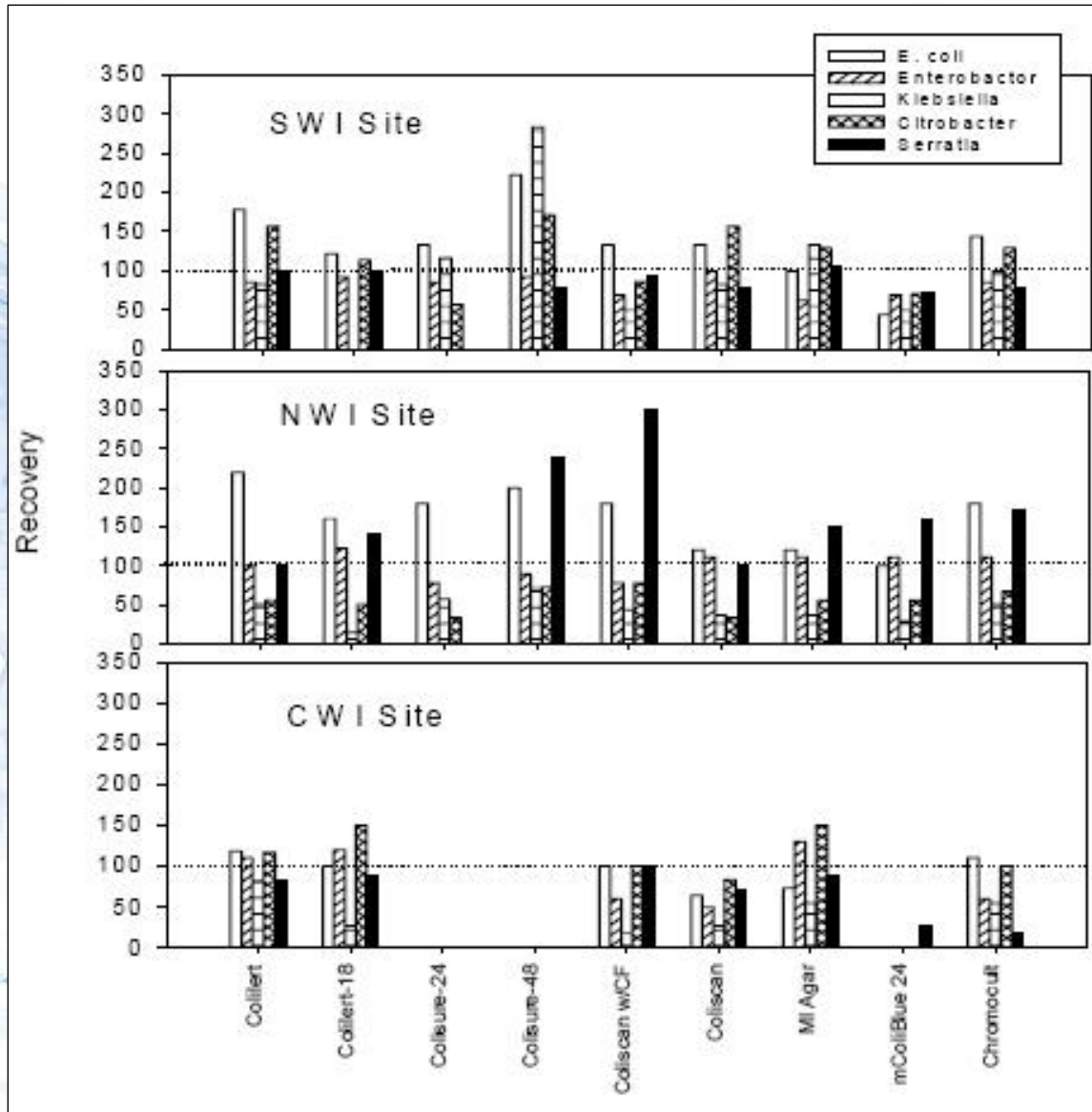
Simple Detection of total coliform and *E. coli*

- 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.

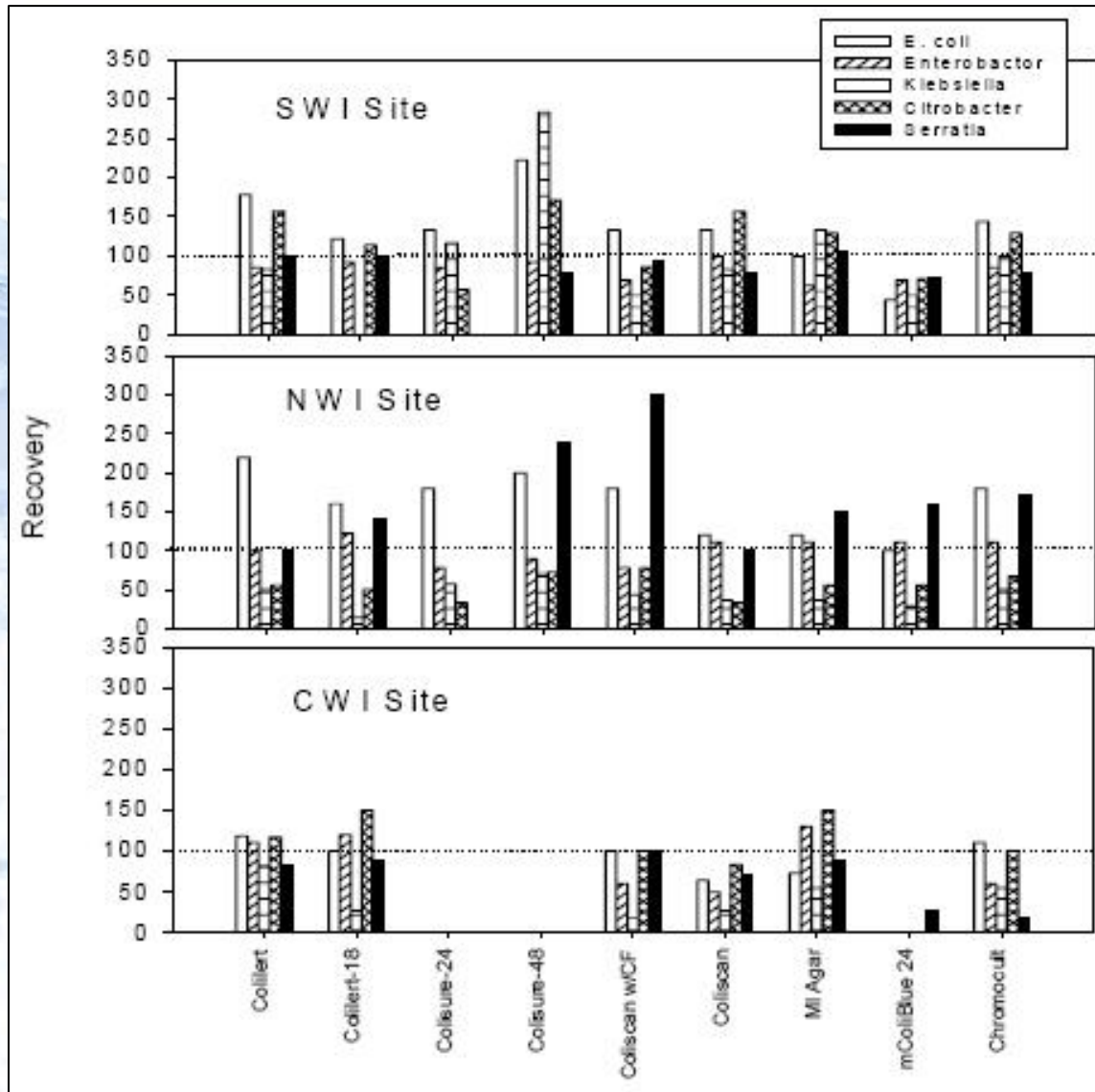
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Chemical characteristics of the groundwater sampling sites

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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 ssp.* - Suppression Objective

- Difference observation in products ability to suppress *Aeromonas ssp.*
- No obvious pattern to each products inability to suppress *Aeromonas ssp.*
- Variability between strains of *Aeromonas ssp.*
- Product inconsistencies observed.

Product Ability to Suppress *Aeromonas* spp. – Site II

	Aeromonas spp.			Strain #1			Aeromonas spp.			Strain #2		
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Coliscan w/CF	-	-	-	-	-	-	-	+	+	+	+	+
Coliscan	-	-	-	-	+	+	-	-	-	+	+	+
MI Agar	-	-	-	-	-	-	+	+	+	+	+	+
mColiBlue 24	-	-	-	-	-	-	+	+	+	+	+	+
Chromocult	+	+	+	+	+	+	+	+	+	+	+	+
Readycult	-	-	-	-	-	-	-	-	-	-	-	-
E*Colite-28	-	-	-	-	-	-	-	+	+	+	+	+
E*Colite-48	+	+	+	+	+	+	+	+	+	+	+	+
Colitag	-	-	-	-	-	-	-	-	-	+	+	-

Analysis of *Aeromonas ssp.* - Suppression Objective (continued)

- The chemical characteristics appeared to have no effect on each products ability or inability to suppress *Aeromonas ssp.*
- With exception of Colilert-18 and ReadyCult Coliforms 100 all methods at some point were unable to completely suppress *Aeromonas ssp.*

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 ssp.*

Future Research Goals

- Further research intent is to generate similar data using organisms cultured from a treated public water system.
- Looking further as 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

- Water Resources Institute – University of Wisconsin System portion of the Wisconsin Groundwater Research Program through the Water Resources Institute.
- Wisconsin State Laboratory of Hygiene – RJ Messling, Linda Peterson, Becky Leidner and Archie Degnan

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