2022 Environmental Measurement Symposium

Apply US-EPA Wastewater & Ambient Water ATP Guideline to Validate Colitag™ for Enumeration of *E. coli* and Fecal Coliform Bacteria

***Dr. Preetha Biswas**, Director of Microbiology, R&D*

Co-Author: Dr. Lei Zhang, Principal Scientist, Microbiology R&D

Outline

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Background

For Wastewater Testing

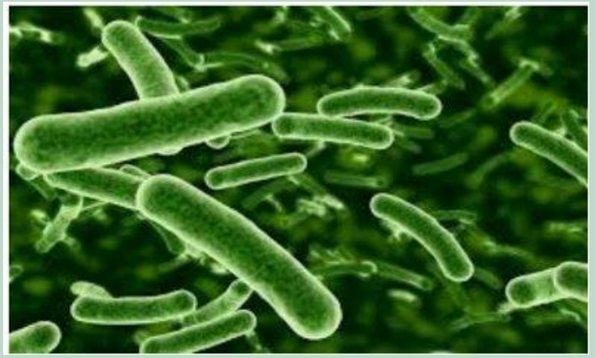
- Clean Water Act (CWA)
  - Established a national commitment to restore and maintain integrity of the nation's waters
  - Improving the health of rivers, lakes, and coastal waters

- National Pollutant Discharge Elimination System (NPDES) permit program
  - Section 402 CWA
  - Regulating point sources that discharge pollutants to waters

Background

For Wastewater Testing

- Microbiological water pollution
- Indicators of microbial water safety
- Commonly used indicator bacteria for water fecal contamination
  - Fecal coliform bacteria
  - *E. coli*
  - *Enterococci*



Colitag

Potable water testing

- Colitag™ originally designed for simultaneous detection of *E. coli* and Total Coliforms in drinking water.
- Colitag for Drinking Water testing is validated both for qualitative and quantitative. Works in presence/absence method, or most probable number-based methodology.

Colitag – Overview

Potable water testing

Colitag medium and method using multi-well MPNTray™ for enumeration of *E. coli* and fecal coliforms.

- Detection is based on the presence of two enzymes
  - ß-galactosidase
    - Coliforms, yellow
  - ß-glucuronidase
    - *E. coli*, fluorescence

- Quantification by most probable number (MPN) method using the number of positive versus negative wells
  - Multi-well tray (97-well)

Objective

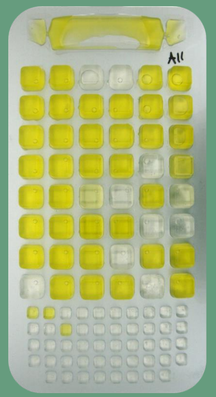
For Wastewater Testing

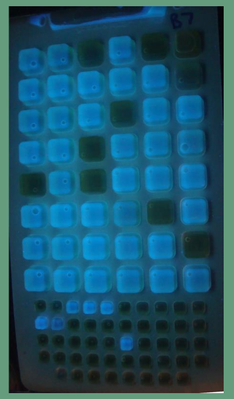
- EPA Microbiological ATP Protocol and Methods to be followed as guideline to evaluate the performance of Colitag medium using multi-well MPNTray, for enumeration of *E. coli* and Fecal Coliform Bacteria in wastewater

ATP Study Requirement

Wastewater Testing

- Follow the Alternate Test Procedure (ATP) guidelines
- Collaboration with EPA coordinator and advisors for review and advice on study proposal – and approval of the method





ATP Study Requirement

Wastewater Testing

Side-by-Side Comparison

- Parallel testing of the ATP and the reference method
- Ten sewage samples from geographic diverse wastewater plant locations
  - Secondary effluent spiked into final sewage targeting to achieve a 20-200 MPN/100 mL sample
- Twenty replicates for each sample to be evaluated

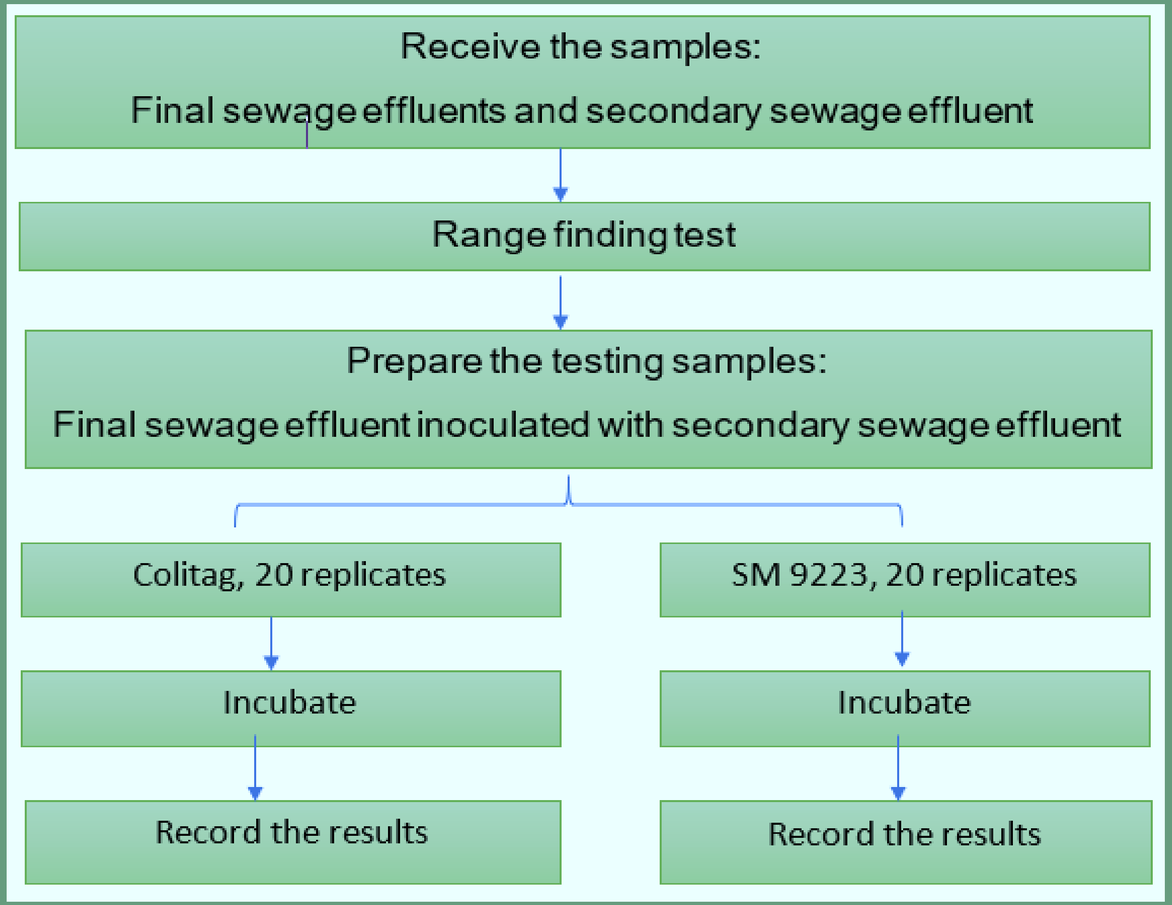
Side-by-Side Comparison ATP Study Plan

Wastewater Testing

Sample source		Replicates	Target Analysis	Analysis method	Minimum comparability results
Type	Number				
Wastewater	10	20	FC	SM 9223	200
				Colitag™	200
	10	20	*E. coli*	SM 9223	200
				Colitag™	200

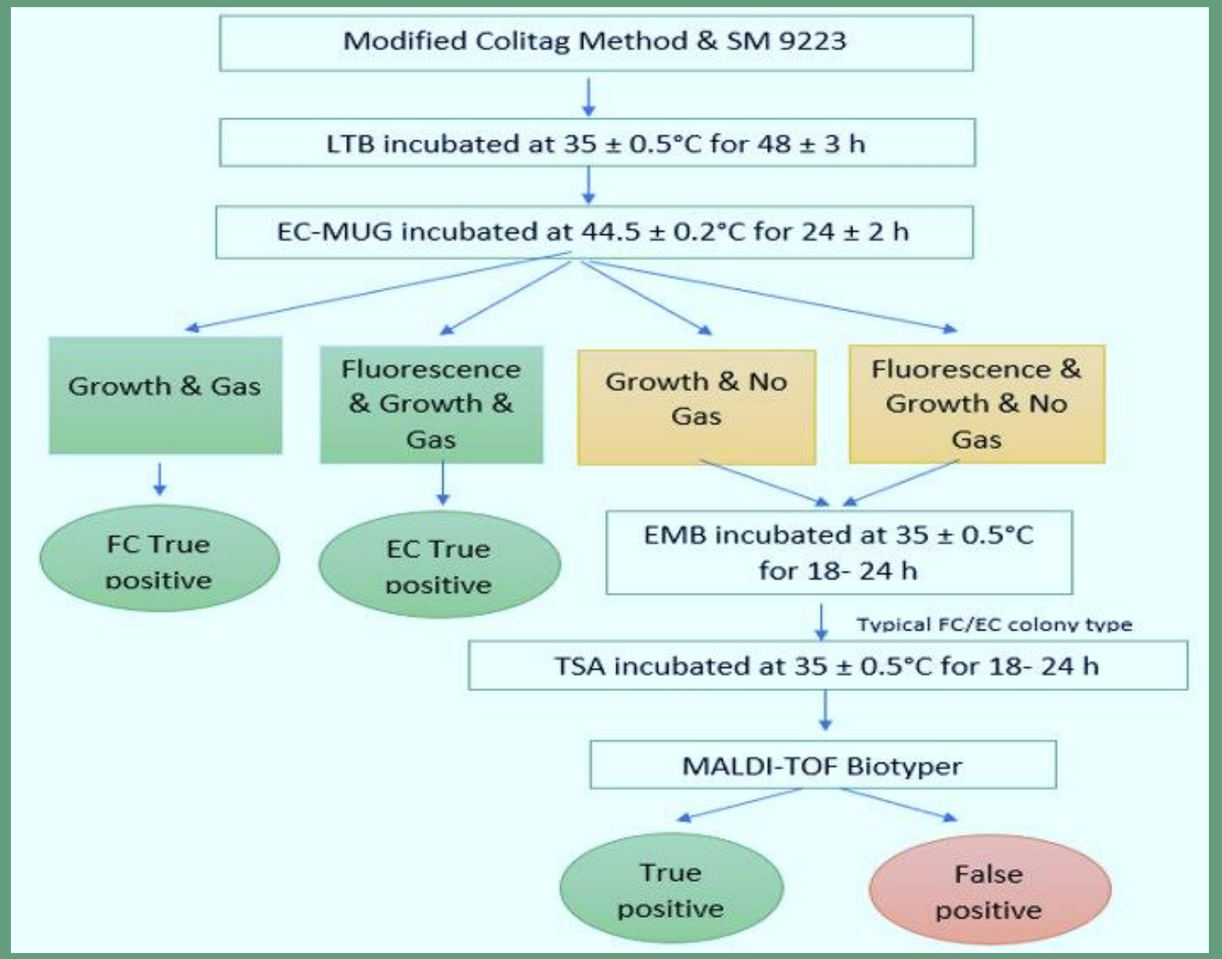
Side-by-Side Comparison Study Flow Chart

Wastewater Testing



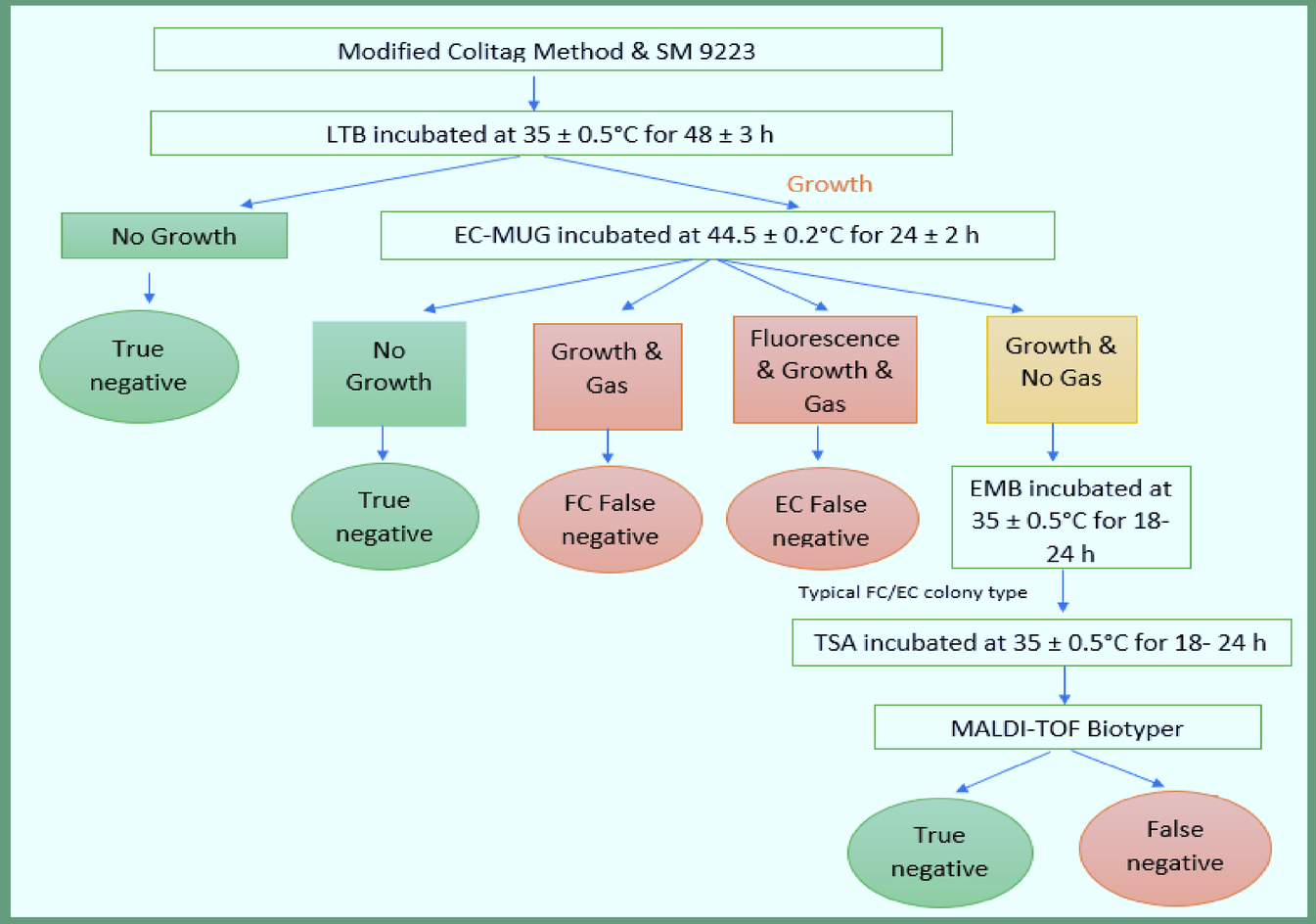
Confirmation Flow Chart for Positive Results

Wastewater Testing



Confirmation Flow Chart for Negative Results

Wastewater Testing



Internal Preliminary Test – Recovery of *E. coli*

Colitag for Wastewater Testing

	Test 1, n=20		Test 2, n=20	
	Average counts per 100 mL	RSD (%)	Average counts per 100 mL	RSD (%)
Colitag	48	20.51	22	23.44
SM 9223B	55	14.23	26	26.66
1603	46	16.92	19	33.76

Verification	Colitag	SM 9223B	1603
Sensitivity	94.7%	86.4%	90%
Specificity	90.5%	94.4%	100%

Internal Preliminary Test – Recovery of FC

Colitag for Wastewater Testing

	Test 1, n=20		Test 2, n=20	
	Average counts per 100 mL	RSD (%)	Average counts per 100 mL	RSD (%)
Colitag	23	21.00	78	18.99
SM 9223B	13	33.56	75	17.28
SM 9222D	24	34.012	66	14.30

Verification	Colitag	SM 9223B	SM 9222D
Sensitivity	100%	95.2%	100%
Specificity	100%	100%	100%

Internal Data: Inclusivity Testing

Colitag Specificity

Inclusivity Organisms		Inoculum (CFU/100 mL)	ONPG (18-24h, 35°C)	MUG (18-24h, 35°C)
• *Citrobacter freundii*	ATCC 6879	21	Positive	Negative
• *Citrobacter freundii*	ATCC 8090	9	Positive	Negative
• *Cronobacter sakazakii*	ATCC 29004	52	Positive	Negative
• *Enterobacter amnigenus*	ATCC 51818	43	Positive	Negative
• *Escherichia coli*	ATCC 8739	18	Positive	Positive
• *Escherichia coli*	ATCC 25922	26	Positive	Positive
• *Escherichia coli*	CDC 984	88	Positive	Positive
• *Escherichia coli*	ATCC 11775	50	Positive	Positive
• *Escherichia coli*	ATCC 11229	20	Positive	Positive
• *Hafnia alvei*	ATCC 51815	42	Positive	Negative
• *Hafnia paralvei*	FSL-W4-0268	47	Positive	Negative
• *Klebsiella oxytoca*	ATCC 2170	28	Positive	Negative
• *Klebsiella pneumoniae*	ATCC 13883	21	Positive	Negative
• *Klebsiella pneumoniae*	ATCC 27736	16	Positive	Negative
• *Serratia marcescens*	ATCC 8100	20	Positive	Negative

Internal Data: Exclusivity Testing

Colitag Specificity

Exclusivity Organisms		Inoculum (CFU/100 mL)	ONPG (48h, 35°C)	MUG (48h, 35°C)
• *Aeromonas hydrophilia*	ATCC 35654	9.1 x 10^5	Negative	Negative
• *Aeromonas hydrophilia*	ATCC 7966	4.0 x 10^6	Negative	Negative
• *Acinetobacter baumannii*	ATCC 19606	5.4 x 10^5	Negative	Negative
• *Alcaligenes faecalis*	ATCC 925	1.0 x 10^6	Negative	Negative
• *Enterococcus faecalis*	ATCC 29212	1.9 x 10^6	Negative	Negative
• *Kocuria rizophilia*	ATCC 9341	6.7 x 10^5	Negative	Negative
• *Pseudomonas aerugniosa*	ATCC 9027	2.3 x 10^6	Negative	Negative
• *Pseudomonas aerugniosa*	ATCC 10145	2.8 x 10^6	Negative	Negative
• *Staphylococcus aureus*	ATCC 25923	3.3 x 10^5	Negative	Negative
• *Bacillus subtilis*	ATCC 9372	2.7 x 10^4	Negative	Negative
• *Bacillus subtilis*	ATCC 6633	4.3 x10^5	Negative	Negative

Side-by-Side Comparison ATP Study Plan - Recap

Wastewater Testing

Sample source		Replicates	Target Analysis	Analysis method	Minimum comparability results
Type	Number				
Wastewater	10	20	FC	SM 9223	200
				Colitag™	200
	10	20	*E. coli*	SM 9223	200
				Colitag™	200

ATP Study Side-by-Side Comparison

Wastewater Testing

Review of study result:

- Mean recovery for each matrix

$$\%\ Recovery = \frac{Result - Background}{Spike} * 100\ \%$$

Where,
Result = the amount recovered from the sample after spiking,
Spike = the estimated amount spiked into the sample, and
Background = the estimated background amount measured in the sample prior to spiking.

- Precision: RSD

$$RSD = \frac{SD}{Mean} * 100\ \%$$

Where,
SD = the standard deviation of all recovered amounts
Mean = the mean of all recovered amounts

ATP Study Side-by-Side Comparison

Wastewater Testing

Review of study result:

- False negative rates

		Method		
		New	Reference	Total
Result	True +	TP_1	TP_2	$TP_1 + TP_2$
	False -	FN_1	FN_2	$FN_1 + FN_2$
	Total	TP_1+FN_1	$TP_2 + FN_2$	$TP_1 + TP_2 + FN_1 + FN_2$

- False positive rates

		Method		
		New	Reference	Total
Result	False +	FP_1	FP_2	$FP_1 + FP_2$
	True -	TN_1	TN_2	$TN_1 + TN_2$
	Total	$FP_1 + TN_1$	$FP_2 + TN_2$	$FP_1 + FP_2 + TN_1 + TN_2$

- QC Acceptance Criteria-Based

  Method Blank, Positive Control and Media Sterility

Thanks to the EPA Team

Thank you for listening

Get in touch

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