



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

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

Co-Authors:

Dr. Lei Zhang, Principal Scientist, Microbiology R&D

Dr. Rob Donofrio, VP Research and Development

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Overview

Symposium

Collaborative Efforts to Improve Environmental Monitoring

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

EPA Microbiological ATP Protocol and Methods followed as guideline to evaluate the performance of Colitag medium using multi-well MPNTray for enumeration of *E. coli* and fecal coliforms in wastewater samples.

Background

For Wastewater Testing

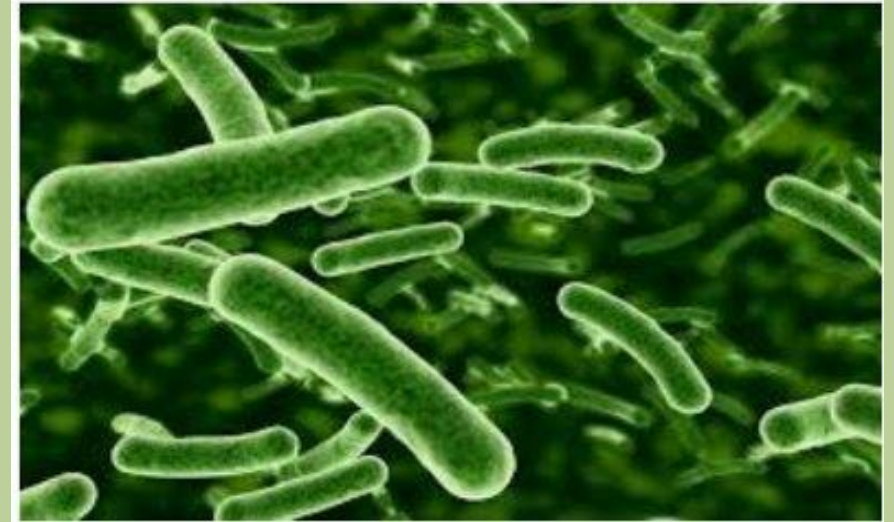
- Clean Water Act (CWA)
 - Established a national commitment to restore and maintain integrity of the nation's water
 - Improving the health of rivers, lakes, and coastal waters
- National Pollutant Discharge Elimination System (NPDES) permit program
 - Section 402 CWA
 - Regulating point sources that 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*



Background

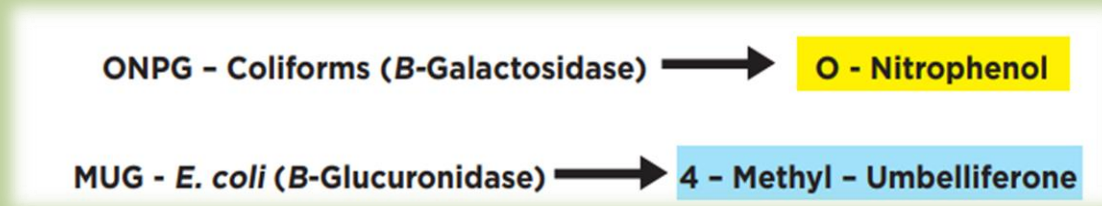
- Modified Colitag™ originally designed for simultaneous detection of *E. coli* and Total Coliforms in drinking water.
- Colitag for Drinking Water testing is a validated method.



Background – Overview

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
 - β -galactosidase
 - Coliforms, yellow
 - β -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

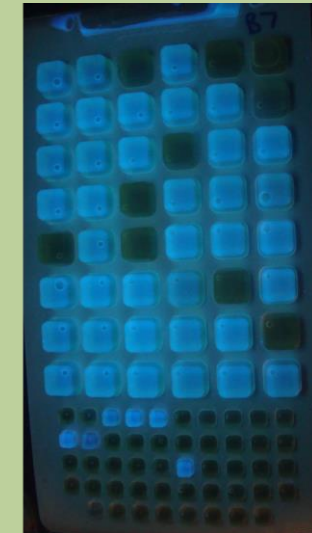
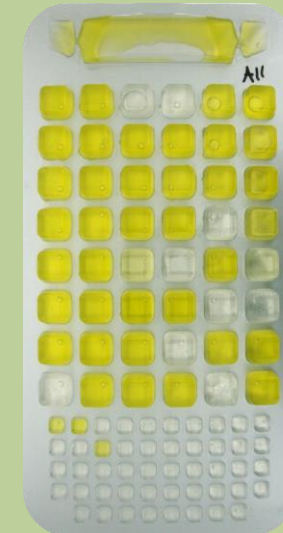
- Validate the Modified Colitag as an ATP for enumeration of *E. coli* and Fecal Coliform Bacteria for Wastewater



ATP Study Plan

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

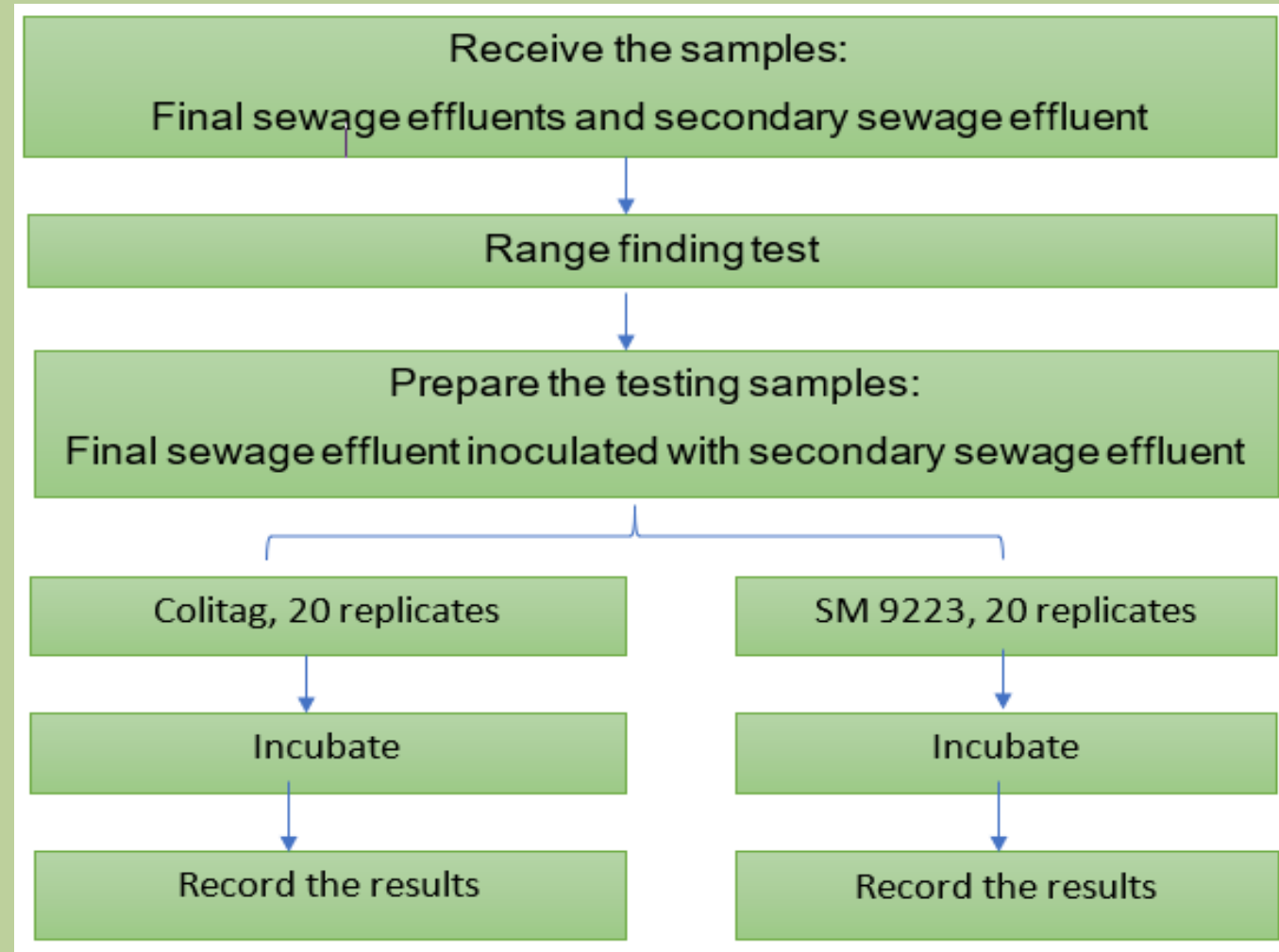
Side-by-Side Comparison ATP Study Plan

Wastewater Testing

Sample source		Replicates	Target Analysis	Analysis method	Minimum comparability results
Type	Number				
Wastewater	10	20	FC	SM 9223	200
				Modified Colitag™	200
	10	20	<i>E. coli</i>	SM 9223	200
				Modified Colitag™	200

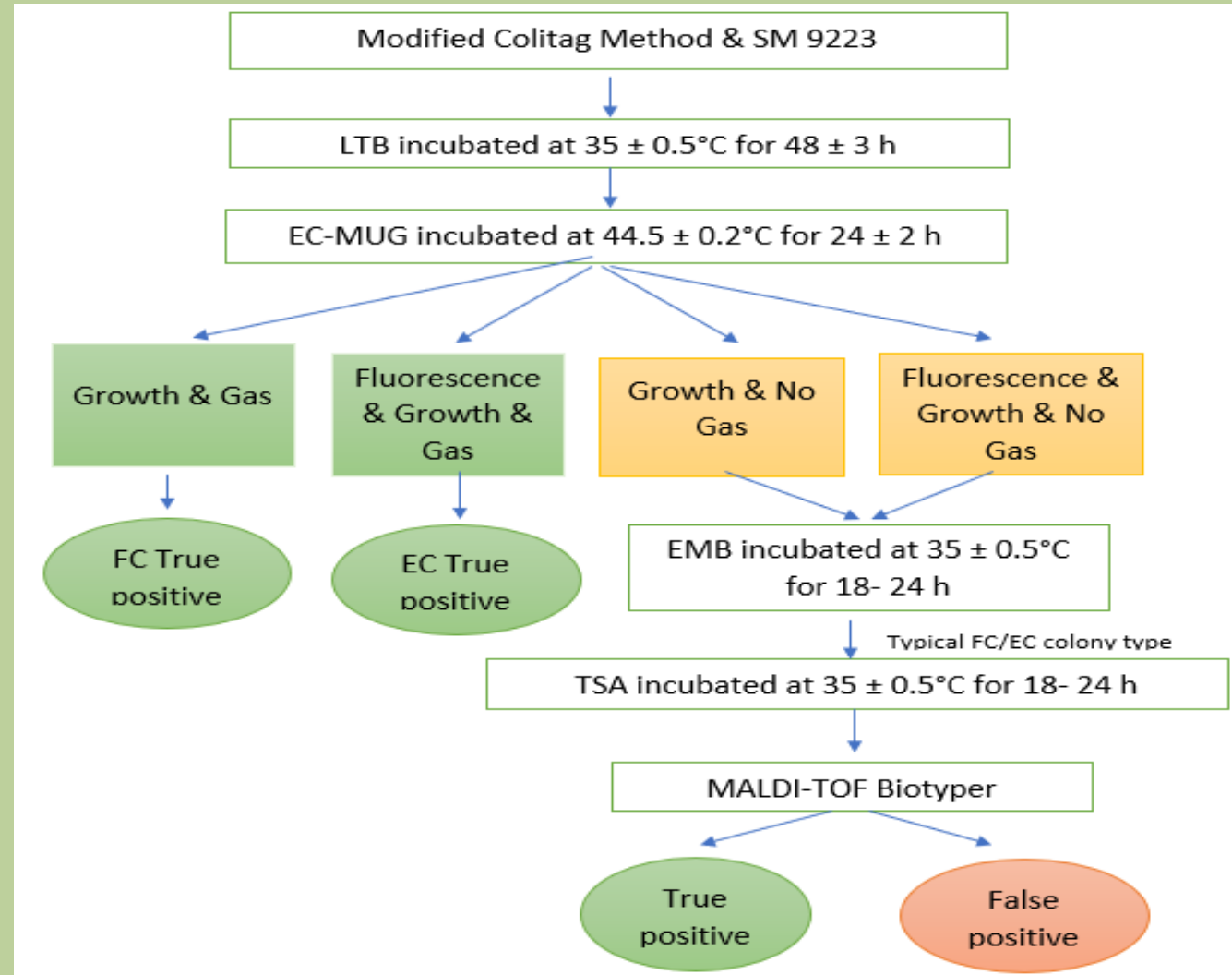
Side-by-Side Comparison Study Flow Chart

Wastewater Testing



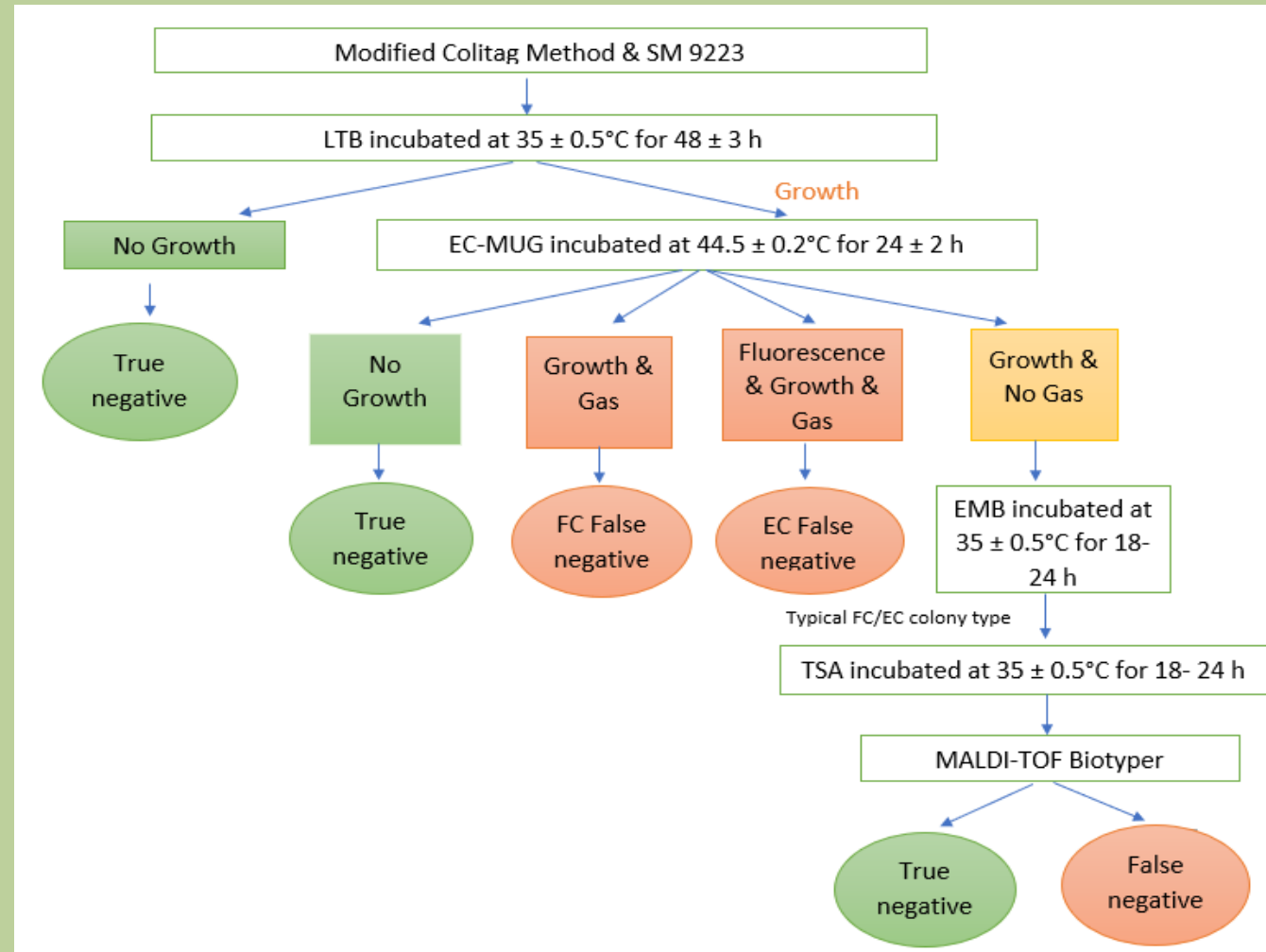
Confirmation Flow Chart for Positive Results

Wastewater Testing



Confirmation Flow Chart for Negative Results

Modified Colitag for Wastewater Testing



Internal Preliminary Test – Recovery of *E. coli*

Modified Colitag for Wastewater Testing

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

Internal Preliminary Test – Recovery of FC

Modified Colitag for Wastewater Testing

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

Internal Preliminary Test – Verification for EC

Modified Colitag for Wastewater Testing

Result	Colitag	SM 9223B	SM 9222D
TP	18	19	20
FP	2	1	0
TN	19	17	17
FN	1	3	3
Overall agreement	92.5	90.0%	92.5%
FP Rate	9.5%	5.6%	0
FN Rate	5.3%	13.6%	10%
Sensitivity	94.7%	86.4%	90%
Specificity	90.5%	94.4%	100%

Sensitivity = $[TP / (TP + FN)] * 100\%$

Specificity = $TN / (TN + FP) * 100\%$

False positive = $1 - \text{Specificity}$

False negative = $1 - \text{Sensitivity}$

Overall Agreement = $[(TP + TN) / TS] * 100\%$

TP = true positives

TN = true negatives

FP = false positives

FN = false negatives

TS = total samples

Internal Preliminary Test – Verification for FC

Modified Colitag for Wastewater Testing

Result	Colitag	SM 9223B	SM 9222D
TP	20	20	20
FP	0	0	0
TN	20	19	20
FN	0	1	0
Overall agreement	100%	97.5%	100%
FP Rate	0	0	0
FN Rate	0	4.8%	0
Sensitivity	100%	95.2%	100%
Specificity	100%	100%	100%

Sensitivity = $[TP/TP + FN]*100\%$

Specificity = $TN/(TN+FP)*100\%$

False positive = $1-\text{Specificity}$

False negative = $1-\text{Sensitivity}$

Overall Agreement = $[(TP+TN)/TS]*100\%$

TP = true positives

TN = true negatives

FP = false positives

FN = false negatives

TS = total samples

Side-by-Side Comparison Study Plan - Recap

Wastewater Testing

Sample source		Replicates	Target Analysis	Analysis method	Minimum comparability results
Type	Number				
Wastewater	10	20	FC	SM 9223	200
				Modified Colitag™	200
	10	20	<i>E. coli</i>	SM 9223	200
				Modified Colitag™	200

ATP Study Side-by-Side Comparison

Wastewater Testing

Methods compared using the following parameters:

- Mean recovery for each matrix
- Precision
- False positive rates
- False negative rates

QC Acceptance Criteria-Based

- Method Blank, Positive Control and Media Sterility Checks

Thanks to the EPA Team

**Thank you
for listening**

To get in touch

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