

Assessment of an Automated E. coli and Fecal Coliform Monitoring System by Comparison with Reference Methods Using the US-EPA ATP Protocol

R. Stephen Brown, L. O'Donnell, School of Environmental Studies and Dept. of Chemistry,
Queen's University, Kingston, ON, Canada K7L 3N6

E.J.P. Marcotte and D. Wilton, TECTA-PDS, Inc., Kingston, ON, K7K 2Y2, Canada

C.R. Fricker, CRF Consulting, Reading, Berkshire, RG1 7NG, United Kingdom



TECTA System for Automated Detection of Bacteria

- Combined *E. coli* (EC)/Total Coliforms (TC) test or *E. coli* (EC)/Fecal Coliforms (FC) test
 - Presence/Absence version of EC/TC approved by US-EPA for drinking water testing
 - Added *E. coli* only test, Enterococcus test
 - Quantitative results provided but not approved
- Goal of current project: US-EPA approval of quantitative EC/FC test



TECTA B16 incubator/detector



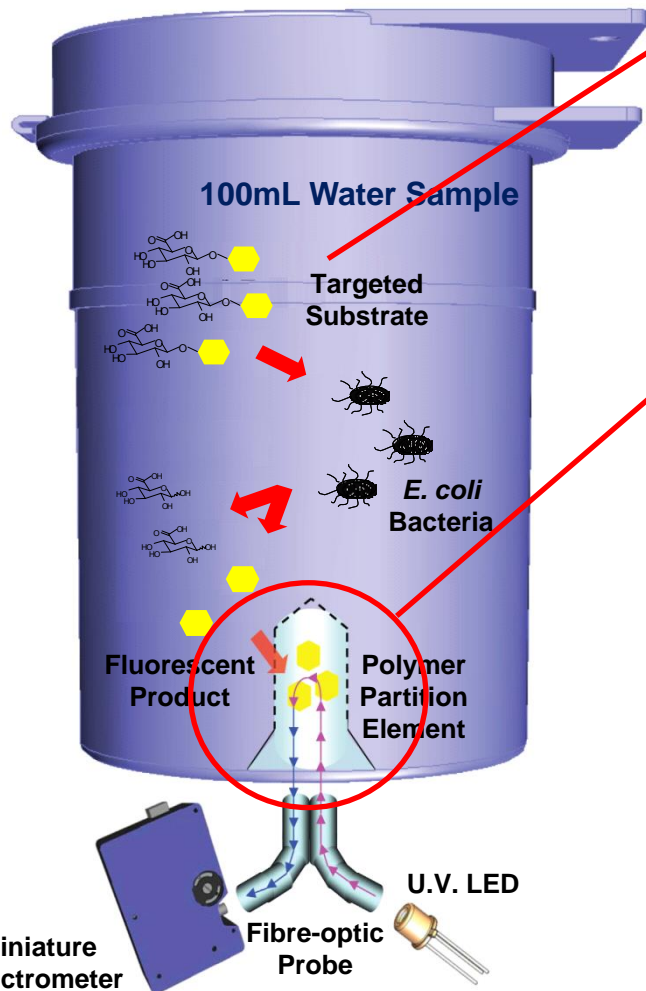
TECTA B4 incubator/
detector



Preloaded, single use
test cartridges

Method overview

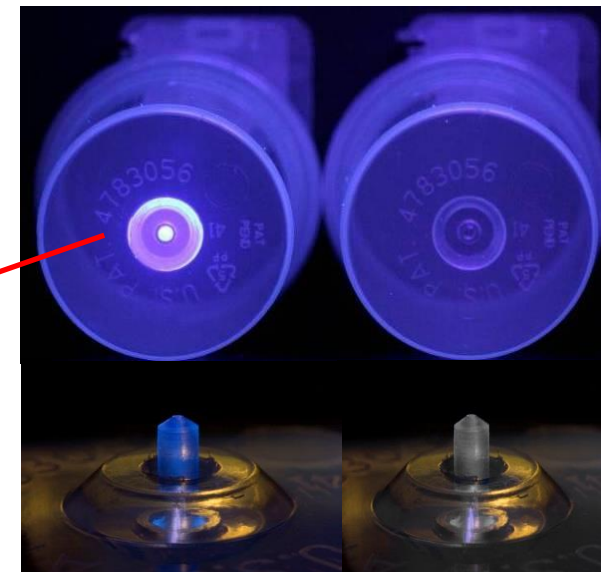
Enzyme-substrate / solution culture method



Detection of indicator enzymes β -D-glucuronidase (*E. coli*) and β -D-galactosidase (Fecal coliforms)
– same as many conventional methods

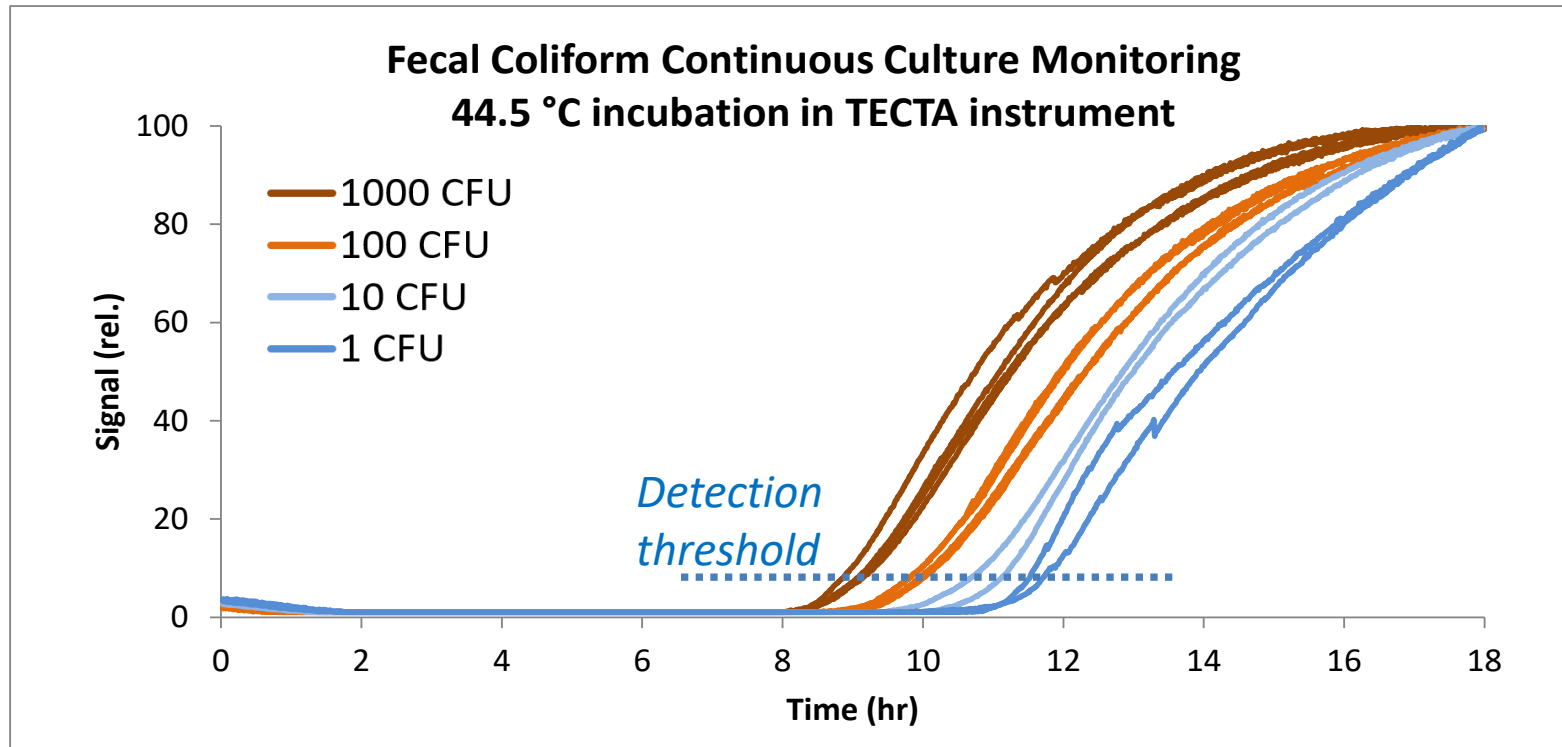
Fluorescent markers extracted from sample matrix into polymer, optical path does not pass through sample

Automated detection of fluorescence in polymer triggers result
- Two indicators detected at separate wavelengths for simultaneous test



Signal Monitoring and Quantitative Analysis

- Signal monitored constantly from polymer in cartridge
 - growth and enzyme expression produce “growth curve”
 - signal onset gives Time-to-Detection (TTD)
 - TTD linearly related to log initial bacteria level



$$C_t = C_0 \cdot 2^{\frac{t-t_l}{t_2}}$$

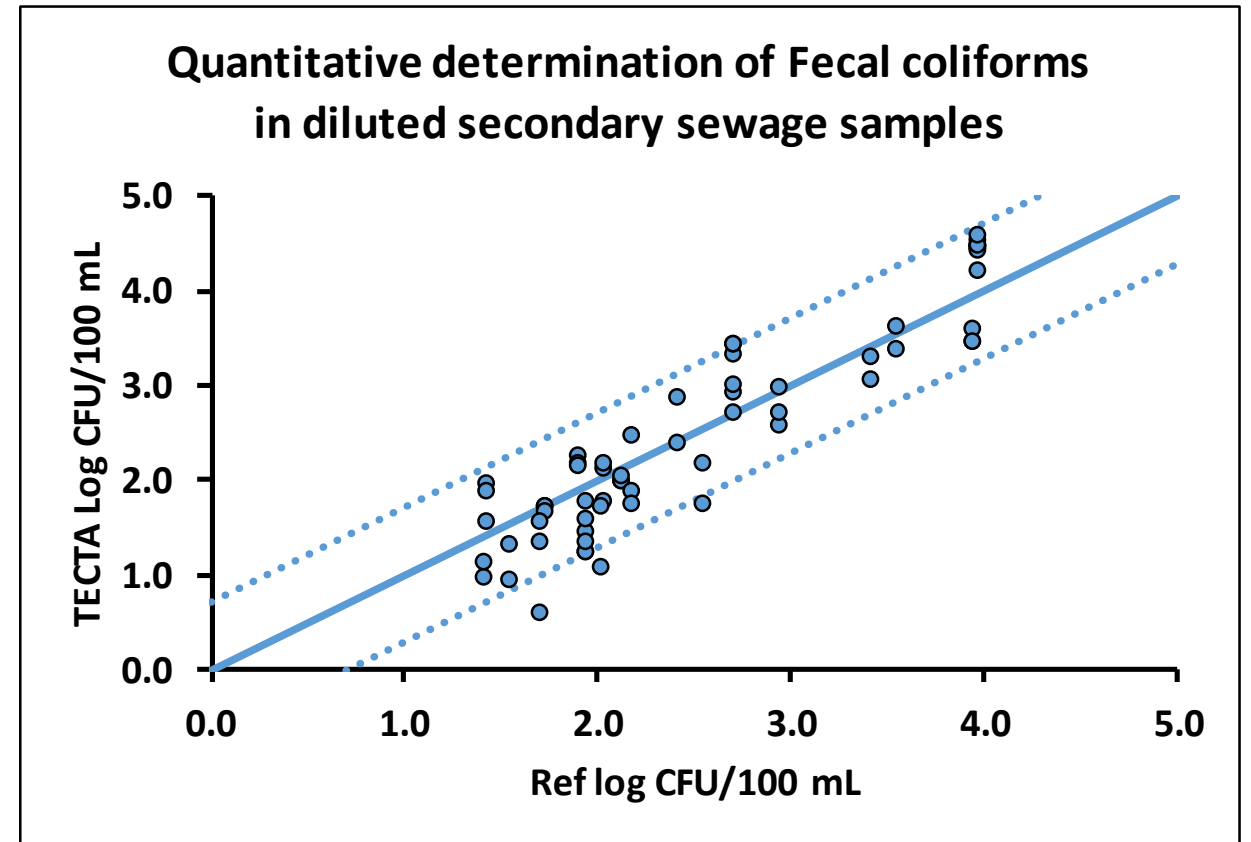
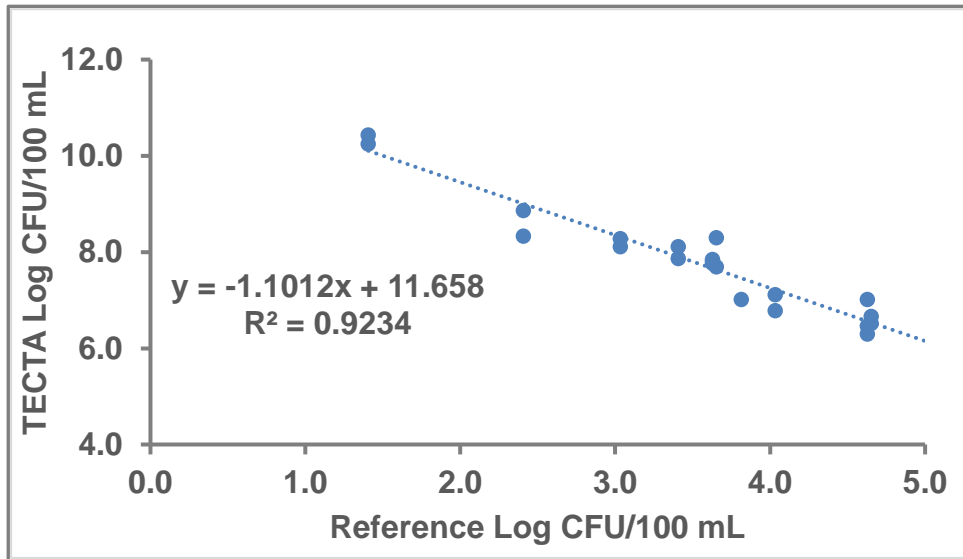
prop. to signal (pointing to C_t)
pop. doubling time (pointing to t_2)
lag phase time (pointing to t_l)

$$C_d = C_0 \cdot 2^{\frac{TTD-t_l}{t_2}}$$

prop. to signal at detection threshold (pointing to C_d)
CFU in orig sample (pointing to C_0)
“Time to detection” (pointing to TTD)
lag phase time (pointing to t_l)

Quantitative Test for Fecal Coliforms

- Calibration using enumerated samples (with chosen reference test)
 - Plot TTD vs Log ref. CFU values
- Validation using independent set of samples
 - Plot of log CFU by TECTA method vs. CFU by reference method



$$TTD = \frac{t_2}{\text{Log}[2]} \text{Log}[C_d] + t_1 - \frac{t_2}{\text{Log}[2]} \text{Log}[C_0]$$

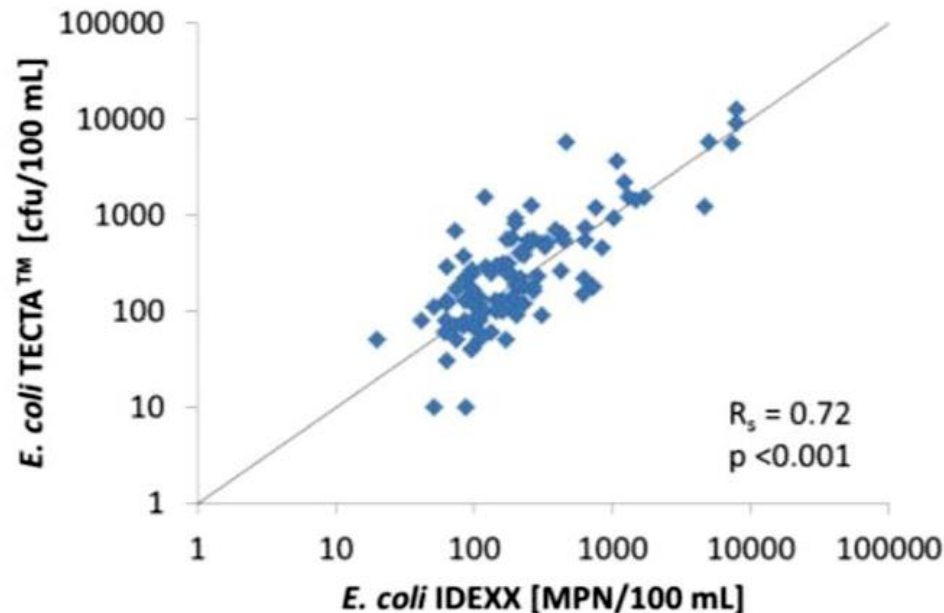
Calib. intercept

Calib. slope

Validation of Quantitative *E. coli* test over time, geography

- Samples from Yarra River, Melbourne, Australia
 - Analysis by McCarthy group, Monash University

Schang *et al.* (2016). Evaluation of techniques for measuring microbial hazards in bathing waters: A comparative study. *PLoS one*, 11(5), e0155848.



- Calibration is fairly robust for wastewater-impacted samples
- *E. coli* calibration in Kingston, ON validated in Melbourne, Australia
- Different reference methods have different calibration functions
- Some users do “custom calibration” and tweak occasionally for optimal performance

- Similar results reported by Prevost group, U. de Montreal

Burnet *et al.* (2019). Autonomous online measurement of β -D-glucuronidase activity in surface water: is it suitable for rapid *E. coli* monitoring?, *Water research* 152: 241-250.

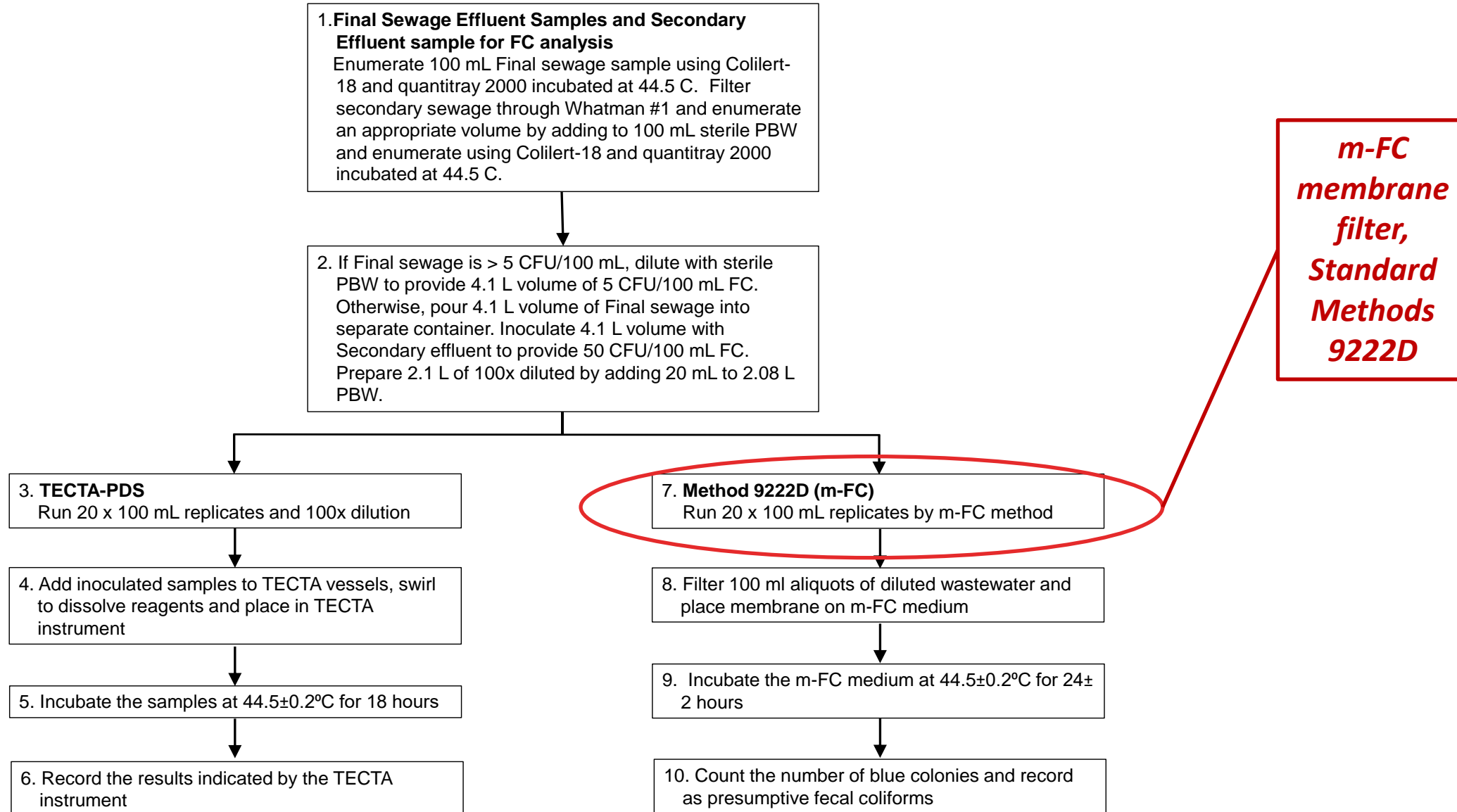
Goal: EPA approval of method for wastewater

- Design Study Plan under Alternate Test Procedure (ATP) protocol
 - https://www.epa.gov/sites/production/files/2015-09/documents/micro_atp_protocol_sept-2010.pdf
 - ATP protocol from 2010, revised version in progress
 - Study Plan reviewed with EPA Clean Water Act ATP coordinator and advisors
- General approach is side-by-side testing with reference method
 - ten final sewage samples from diverse locations across USA
 - if bacteria level < 5 CFU/100 mL, inoculate with secondary sewage to reach 20-60 CFU/100 mL range (20-80 CFU/100 mL for EC)
 - an additional 100-fold dilution prepared to provide negative samples
 - sets of twenty 100 mL replicates run by each method
 - reference methods mFC (SM 9222D for fecal coliforms) and modified m-TEC (US EPA Method 1603 for *E. coli*)

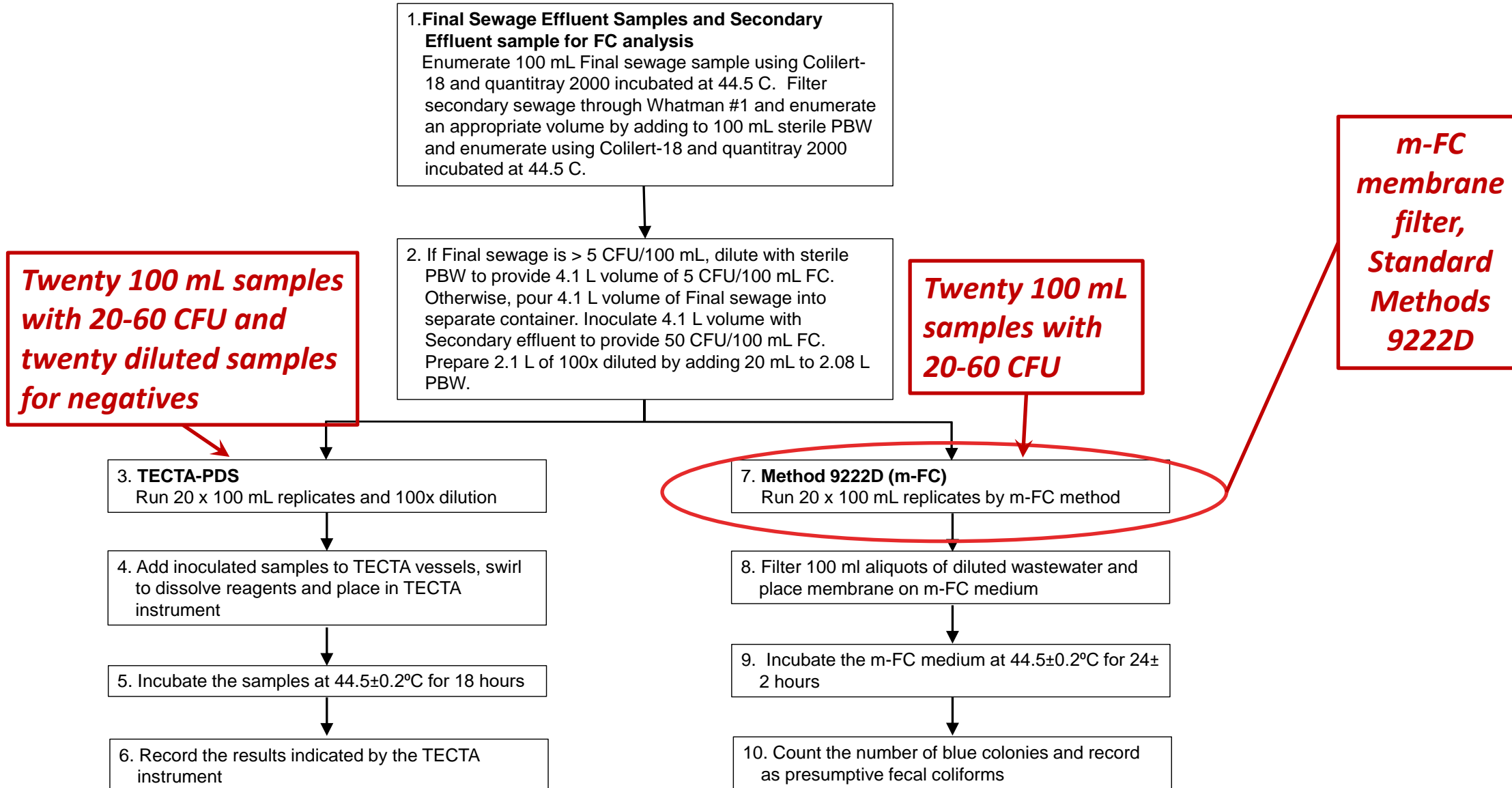
Study Plan details

- Use TECTA for *E. coli* and Fecal Coliforms simultaneously
 - Incubate at 44.5 °C for thermotolerant *E. coli* and coliforms
 - Thermotolerant *E. coli* counts are statistically similar to *E. coli* counts across 35 °C – 44.5 °C range
 - Thermotolerant coliforms are defined as “Fecal Coliforms”
 - TECTA system confirmed to match temperature specifications of typical water bath (± 0.2 °C)
- Selectivity and sensitivity determined through confirmations
 - Confirmation protocol established using standard methods
 - Flow charts developed along with detailed SOP document to help 3rd party lab reproduce planned protocol

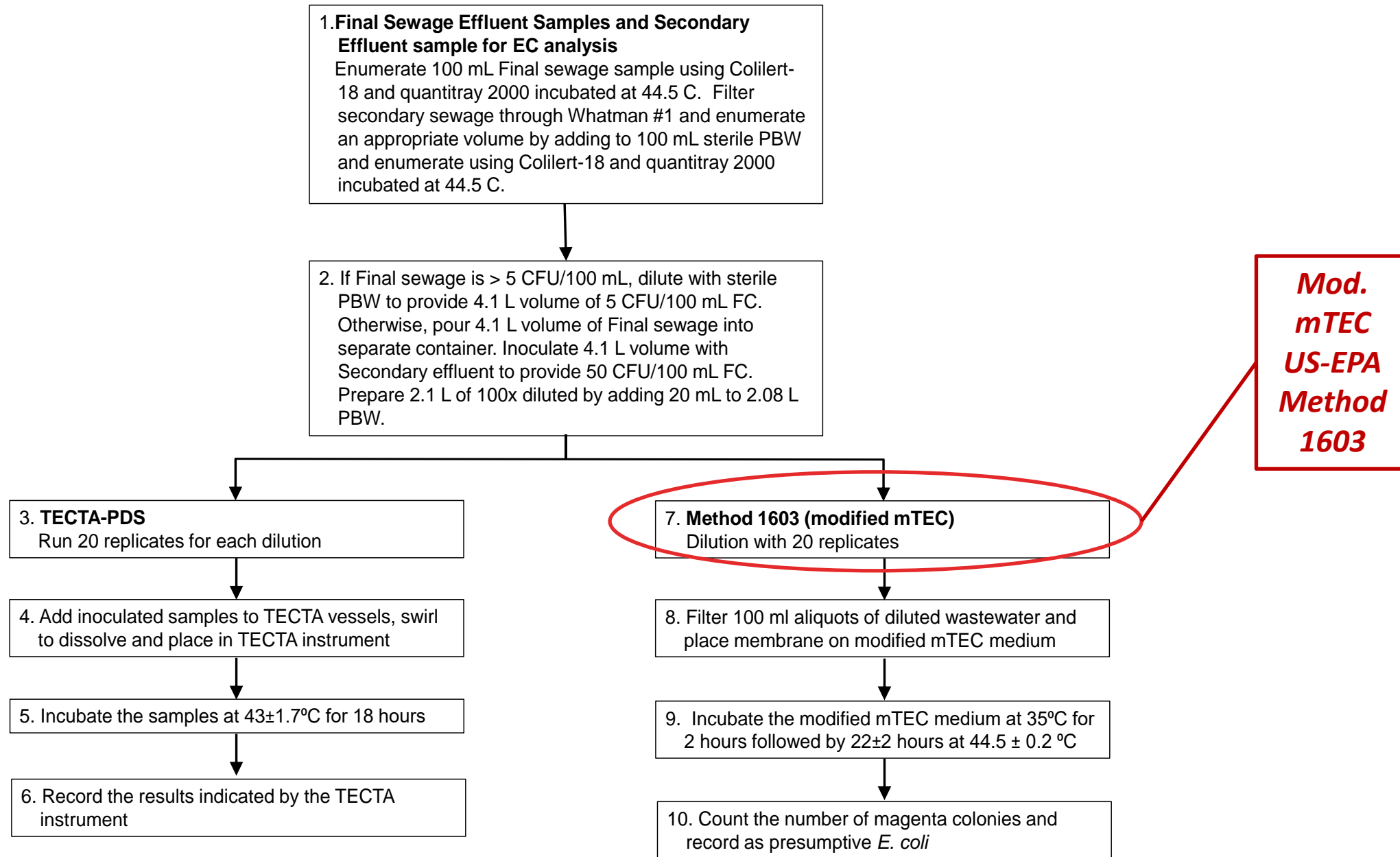
Study Plan – Fecal Coliform Flow Chart



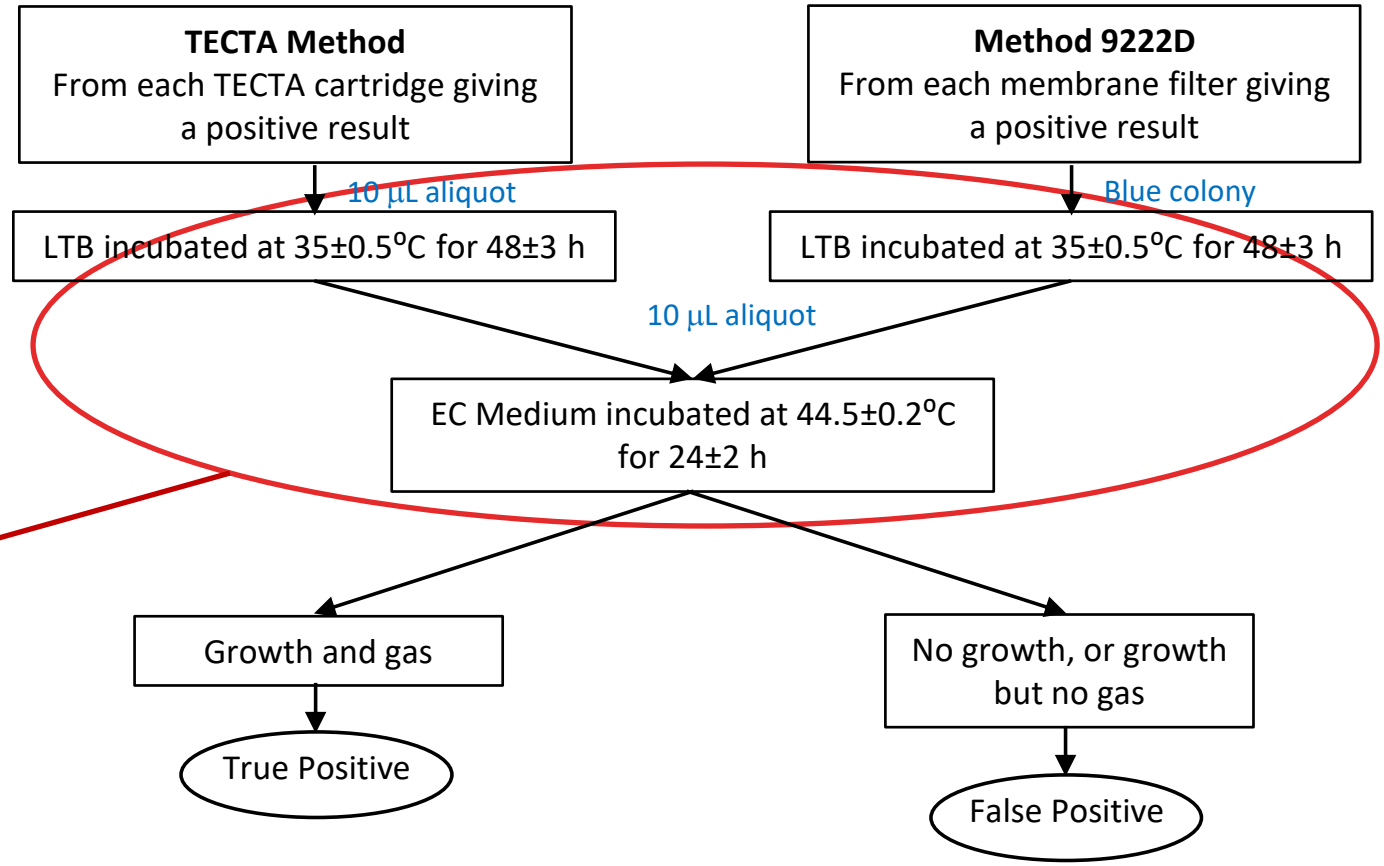
Study Plan – Fecal Coliform Flow Chart



Study Plan – *E. coli* Flow Chart



Fecal Coliform Confirmation Flow Chart

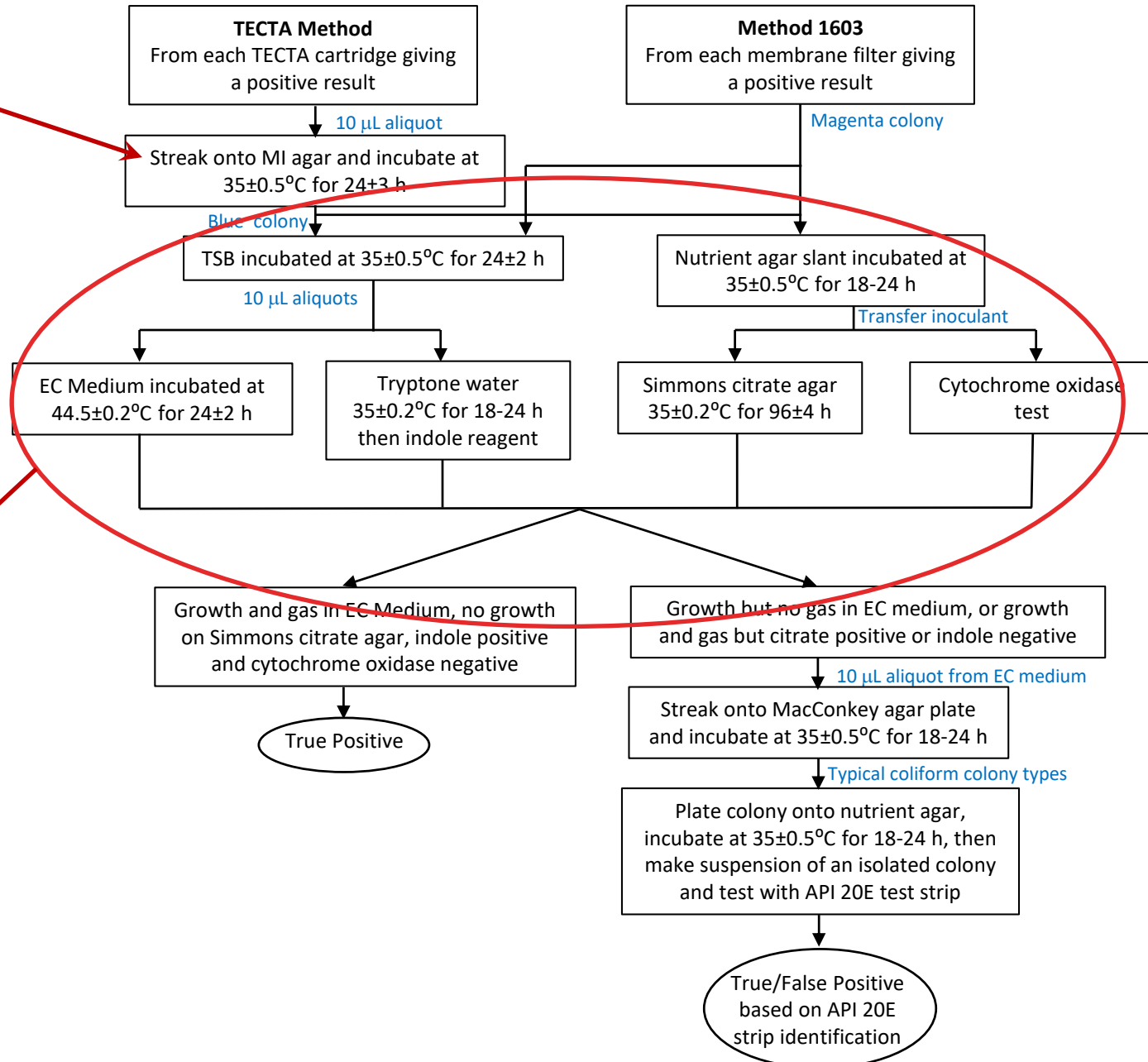


**Key is LTB
then EC
Medium
growth
and gas**

E. coli Confirmation Flow Chart

Recovery on MI agar provides isolates from positive samples (also based on β -D-glucuronidase)

EC Medium + cytochrome oxidase -ve, indole +ve, Simmons citrate -ve



ATP Study

- **3rd party lab contracted to do work, supplied with four TECTA B16 instruments**
 - confirmed protocol is feasible by 1-2 technicians
 - generated preliminary data to confirm training
 - characterized local source of secondary sewage for spiking
- **Final sewage effluents obtained from ten WWTPs**
 - Five in-state, five out-of-state
 - Final sewage all <5 CFU/100 mL, spiked with secondary
 - When source FC and EC levels were similar, could do both with same TECTA sample

ATP Study Results

- *Fecal coliform* (FC) quantitation
 - all positive TECTA samples detected in under 10.6 hours
 - variability of TECTA method higher than mFC (RSD 49% vs 16%)
 - statistically different ($p < 0.05$), but two means well within one Std. Dev.
 - recoveries in expected range, TECTA higher overall recovery

Fecal Coliforms: Results Summary													
	TECTA FC					mFC							
	Mean	Range CFU/100 mL		Std Dev	RSD	Recovery	Mean	Range CFU/100 mL		Std Dev	RSD	Recovery	t-test
	cfu/100 mL	Low	High	cfu/100 mL	(%)	(%)	cfu/100 mL	Low	High	cfu/100 mL	(%)	(%)	
Sample 1	33	5	100	20.3	62%	110%	46	24	62	11.4	25%	155%	0.0142
Sample 2	81	17	194	39.2	49%	131%	64	49	79	8.5	13%	104%	0.0714
Sample 3	52	7	111	30.7	59%	133%	51	28	71	11.9	23%	129%	0.8396
Sample 4	93	32	230	50.7	54%	172%	56	40	74	9.9	18%	103%	0.0026
Sample 5	47	23	78	15.5	33%	88%	62	51	74	7.1	11%	117%	0.0002
Sample 6	14	5	28	6.1	44%	28%	57	44	69	6.4	11%	116%	0.0000
Sample 7	59	14	95	20.9	36%	106%	57	38	73	8.2	14%	103%	0.7665
Sample 8	149	45	314	70.0	47%	296%	47	35	63	6.8	15%	94%	0.0000
Sample 9	119	36	345	70.9	59%	258%	51	37	63	7.1	14%	110%	0.0001
Sample 10	139	59	356	63.1	45%	212%	72	48	88	10.1	14%	111%	0.0000
Average	78.5			38.7	49%	153%	56.3			8.7	16%	114%	0.0349

ATP Study Results

- *E. coli* (EC) quantitation
 - all positive TECTA samples detected in under 9.4 hours
 - variability of TECTA method higher than mod.mTEC (RSD 48% vs 15%)
 - statistically different ($p < 0.05$), but two means well within one Std. Dev.
 - recoveries in expected range, TECTA recovery maybe slightly higher

<i>E. coli</i> : Results Summary													
	TECTA EC						mTEC						
	Mean	Range CFU/100 mL		Std Dev	RSD	Recovery	Mean	Range CFU/100 mL		Std Dev	RSD	Recovery	t-test
	cfu/100 mL	Low	High	cfu/100 mL	(%)	(%)	cfu/100 mL	Low	High	cfu/100 mL	(%)	(%)	
Sample 1	25	10	48	11.4	45%	129%	20	12	32	5.0	25%	104%	0.0823
Sample 2	48	18	76	18.4	39%	64%	83	37	152	20.8	25%	111%	0.0000
Sample 3	85	19	170	34.9	41%	128%	66	48	74	5.8	9%	100%	0.0227
Sample 4	49	8	100	29.5	60%	76%	76	51	90	11.3	15%	118%	0.0005
Sample 5	94	33	220	50.0	53%	101%	94	69	108	10.1	11%	101%	0.9861
Sample 6	46	21	77	15.2	34%	76%	69	50	89	9.0	13%	115%	0.0000
Sample 7	56	13	99	22.6	41%	89%	64	45	79	8.3	13%	102%	0.1358
Sample 8	86	12	173	42.5	50%	165%	50	35	67	9.3	19%	96%	0.0008
Sample 9	102	25	351	72.7	72%	174%	56	47	69	6.5	12%	96%	0.0083
Sample 10	142	53	371	71.9	51%	217%	68	57	86	8.4	12%	105%	0.0001
Average	73.1			36.9	48%	122%	64.6			9.5	15%	105%	0.0349

False positive and False Negative rates

- Confirmation results for 200+ TECTA and reference samples
 - All parameters within expected range for TECTA, mod.mTEC (>90%)
 - mFC reference test had higher false rates, matches previous literature

False Positive Rate

$$= \text{FP}/(\text{TN}+\text{FP}) \times 100\%$$

False Negative Rate

$$= \text{FN}/(\text{TP}+\text{FN}) \times 100\%$$

Sensitivity

$$= \text{TP}/(\text{TP}+\text{FN}) \times 100\%$$

Specificity

$$= \text{TN}/(\text{TN}+\text{FP}) \times 100\%$$

	TECTA FC		mFC	
Sample	FP rate	FN rate	FP rate	FN rate
1	0%	17%	35%	29%
2	0%	0%	6%	14%
3	5%	0%	28%	32%
4	0%	0%	7%	24%
5	0%	0%	14%	6%
6	6%	0%	18%	26%
7	0%	0%	6%	21%
8	14%	8%	0%	29%
9	0%	0%	20%	32%
10	0%	0%	10%	10%
Extra	0%	0%		
Total	1.9%	2.8%	15.3%	22.8%

	TECTA EC		mod.m-TEC	
Sample	FP rate	FN rate	FP rate	FN rate
1	0%	0%	5%	10%
2	0%	0%	9%	0%
3	0%	0%	0%	0%
4	0%	0%	13%	0%
5	13%	0%	5%	10%
6	13%	0%	19%	16%
7	13%	0%	5%	10%
8	0%	7%	0%	17%
9	0%	0%	0%	13%
10	0%	0%	5%	5%
Extra	0%	0%		
Total	4.1%	1.0%	6.6%	8.3%

	TECTA FC	mFC	TECTA EC	mod.m-TEC
Sensitivity	97.2%	77.2%	99.0%	91.7%
Specificity	98.1%	84.7%	95.9%	93.4%

Summary

- **Assessment of TECTA EC and FC quantitative tests for wastewater**
 - confirm TECTA test is easy to do in 3rd party lab
 - results in <11 h for positive samples, precision in expected range
 - TECTA recovery slightly higher than reference methods (esp. for FC)
 - quantitative comparisons favourable, differences between methods within standard deviation ranges
- **Study report and data currently being assessed by US-EPA Wastewater Group**

Acknowledgements

- Funding for this work:



Natural Sciences and Engineering
Research Council Canada
CRD, I2I programs



Province of Ontario
OCE, MRIS, MOECC



Questions?

stephen.brown@chem.queensu.ca

www.tecta-pds.com

