

Implementing the US-EPA Wastewater ATP to Characterize a Rapid, Automated System for Monitoring *E. coli* and Fecal Coliform Bacteria

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TECTA System for Automated Detection of Bacteria

- Originally designed for drinking water testing
 - First test for *E. coli* and Total Coliforms
 - Presence/Absence version approved by US-EPA
 - Added tests for Enterococcus and Fecal Coliforms
 - Quantitative results provided but not approved



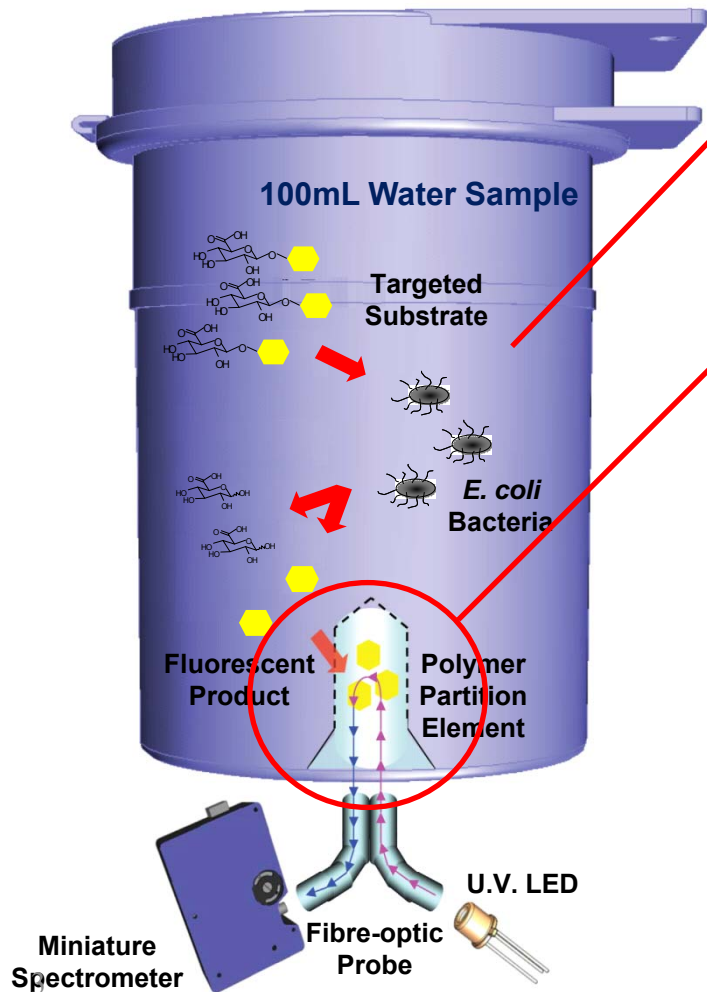
Preloaded, single use test cartridges



TECTA B16 combined incubator/detector

Method overview

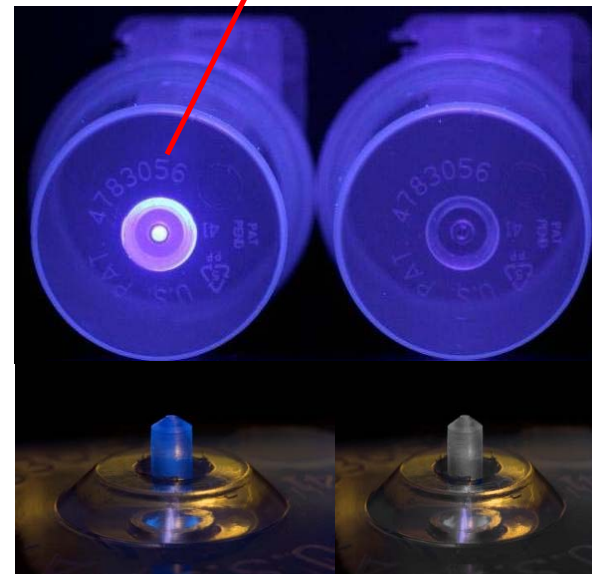
Enzyme-substrate / solution culture method



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

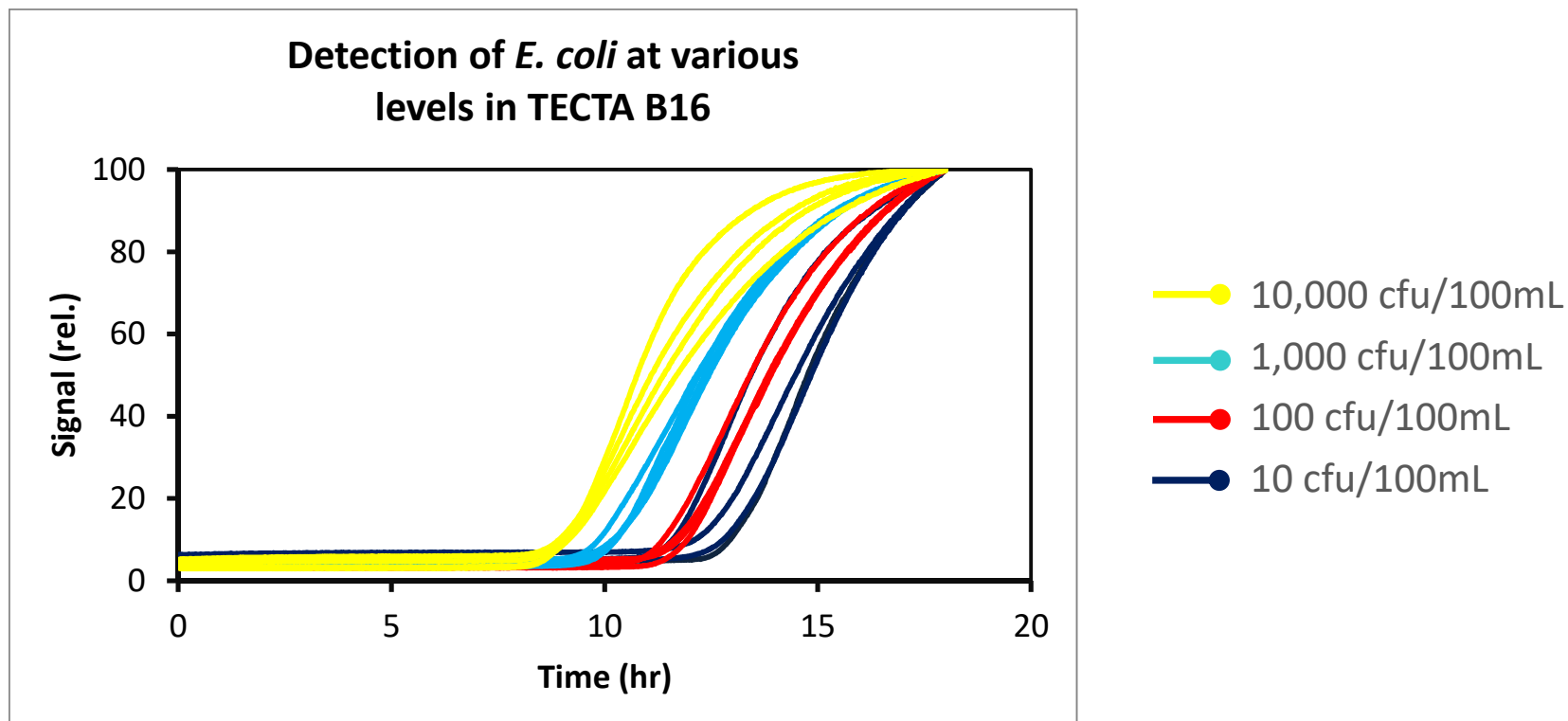
Fluorescent markers extracted from sample matrix into polymer, optical path does not pass through sample

Automated detection of fluorescence in polymer triggers result



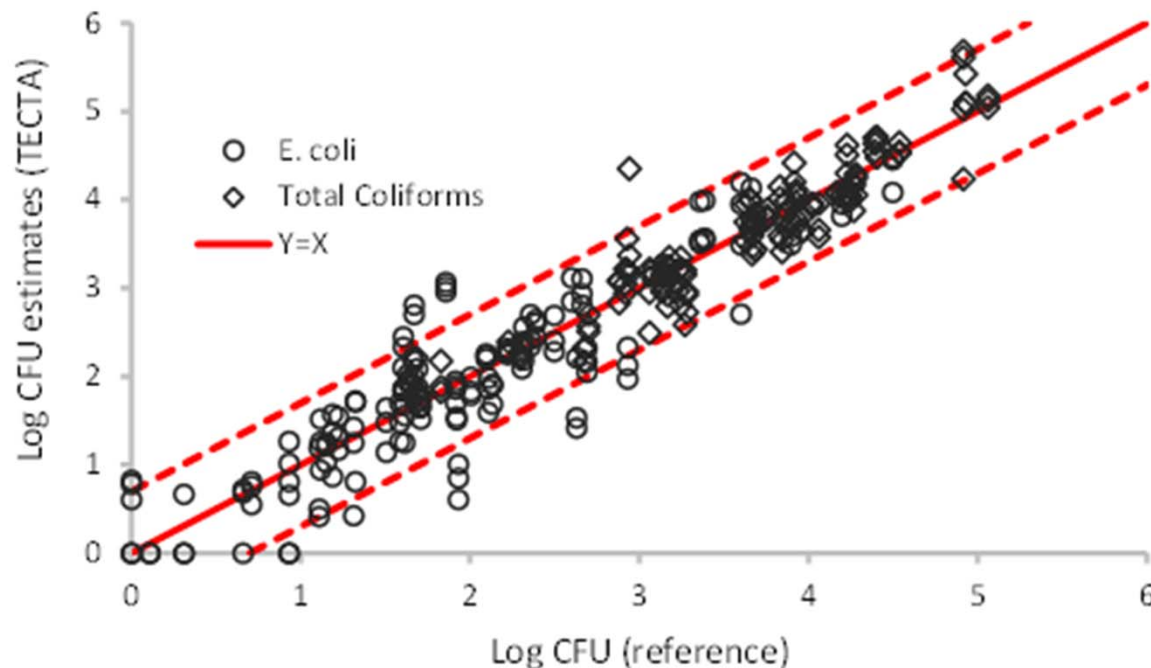
Signal Monitoring and Quantitative Analysis

- 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



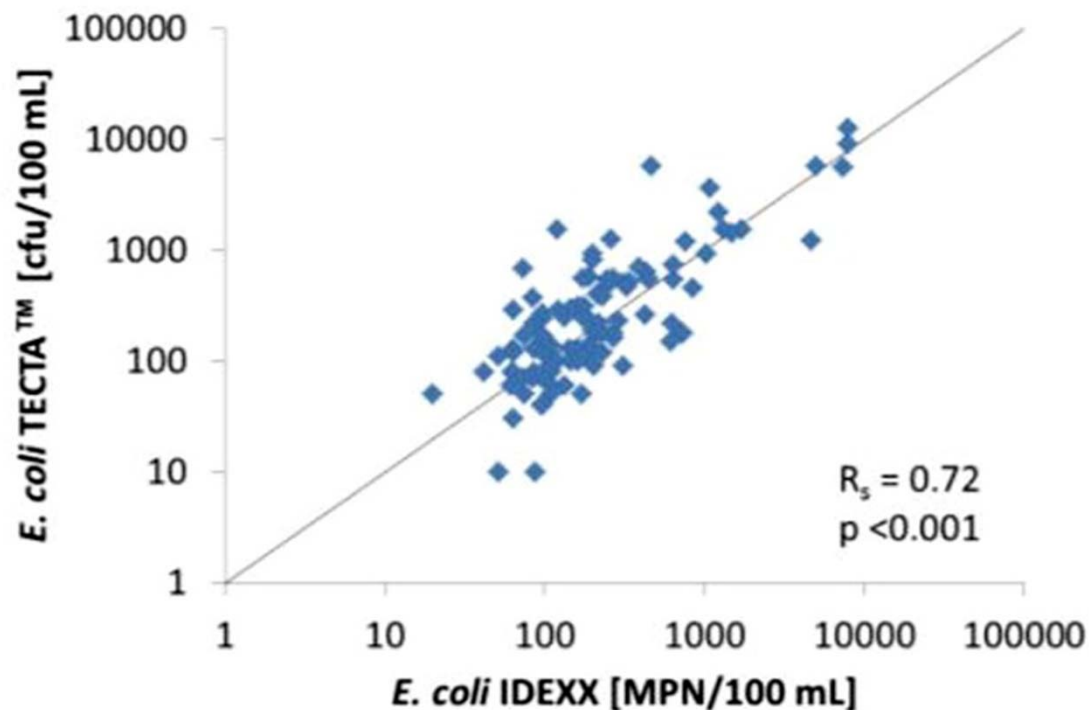
Validation of Quantitative Analysis

- Validated using lake water spiked with sewage
 - *E. coli* and Total Coliforms tested simultaneously
 - Samples run ~1 year after calibration
 - 95% of results within 0.7 log of reference
 - comparable to inter-lab studies using different methods



Validation of Quantitative Analysis

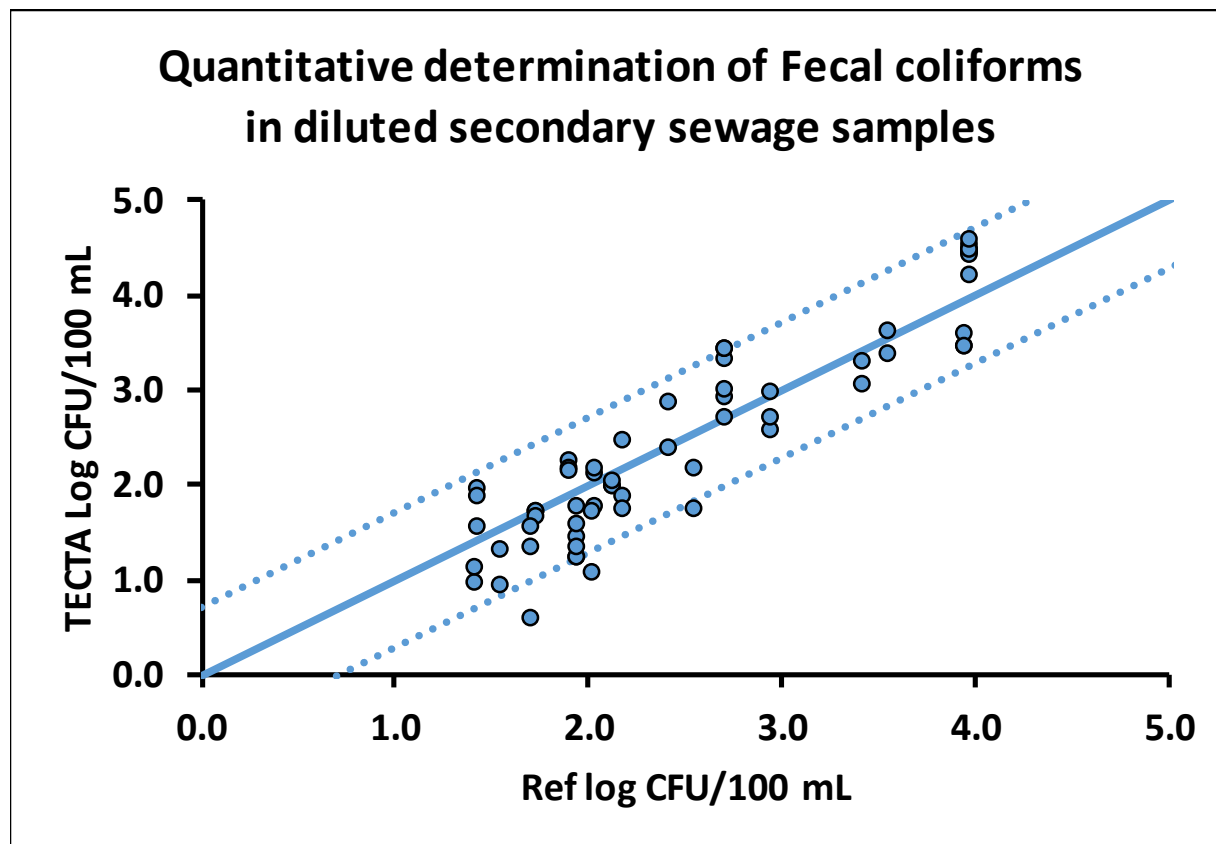
- Samples from Yarra River, Melbourne, Australia
 - Analysis by McCarthy group, Monash University



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

Quantitative Test for Fecal Coliforms

- Total Coliforms test run at 44.5 °C
 - Validation of calibration from previous year
 - Performance similar to Total Coliforms test



Goal: EPA approval of method for wastewater

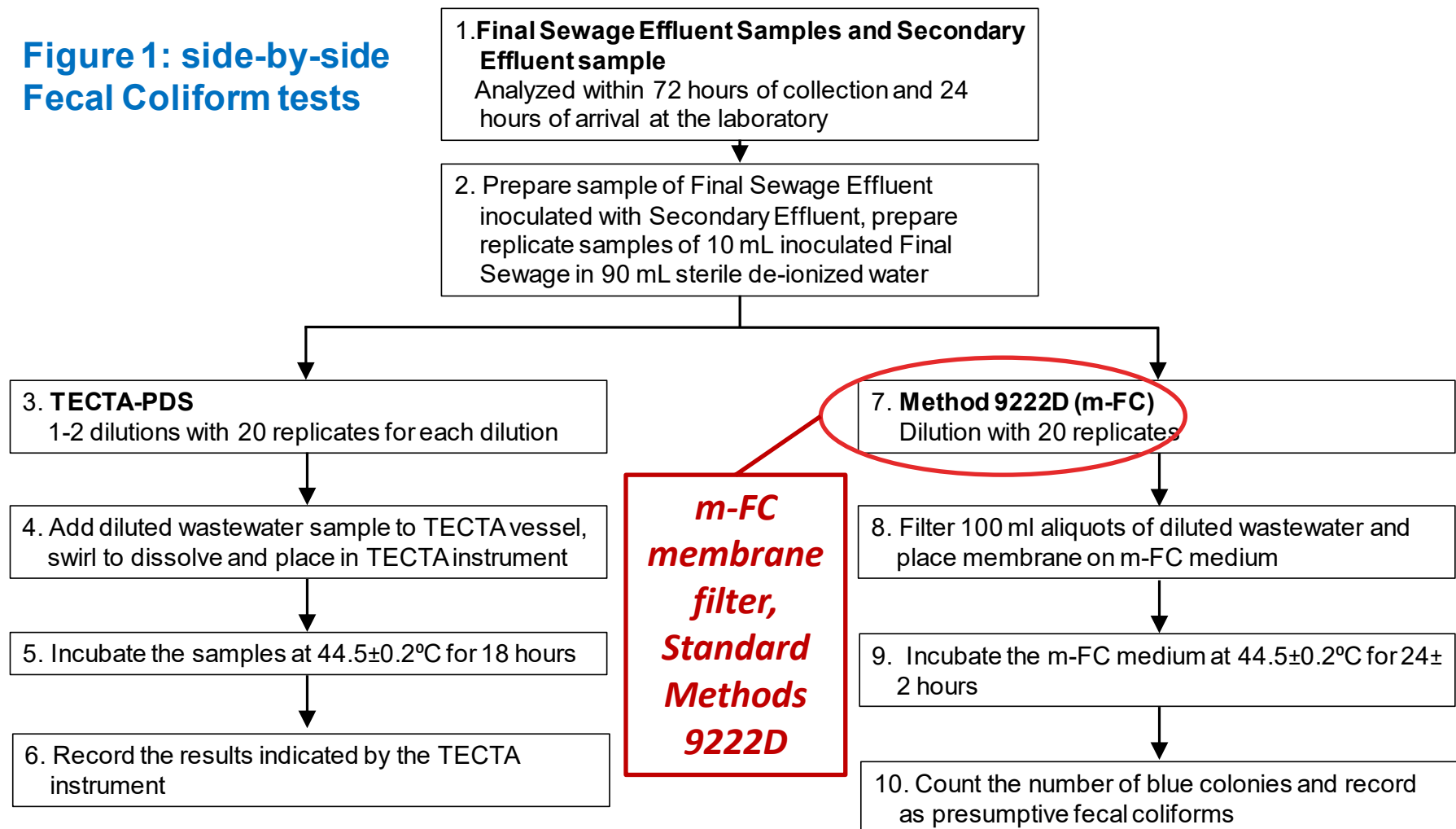
- Design Study Plan under Alternate Test Procedure (ATP) protocol
https://www.epa.gov/sites/production/files/2015-09/documents/micro_atp_protocol_sept-2010.pdf
 - ATP protocol from 2010, revised version planned
 - Study Plan reviewed with EPA Clean Water Act ATP coordinator and advisors
- General approach is side-by-side testing with reference method
 - ten final sewage samples from diverse locations around USA
 - sets of twenty 10 mL replicates run by each method
 - if bacteria levels below 30 CFU in 10 mL sample, spike with secondary sewage effluent (before disinfection)
 - Reference methods mFC (fecal coliforms) and modified m-TEC (*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 coliform 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

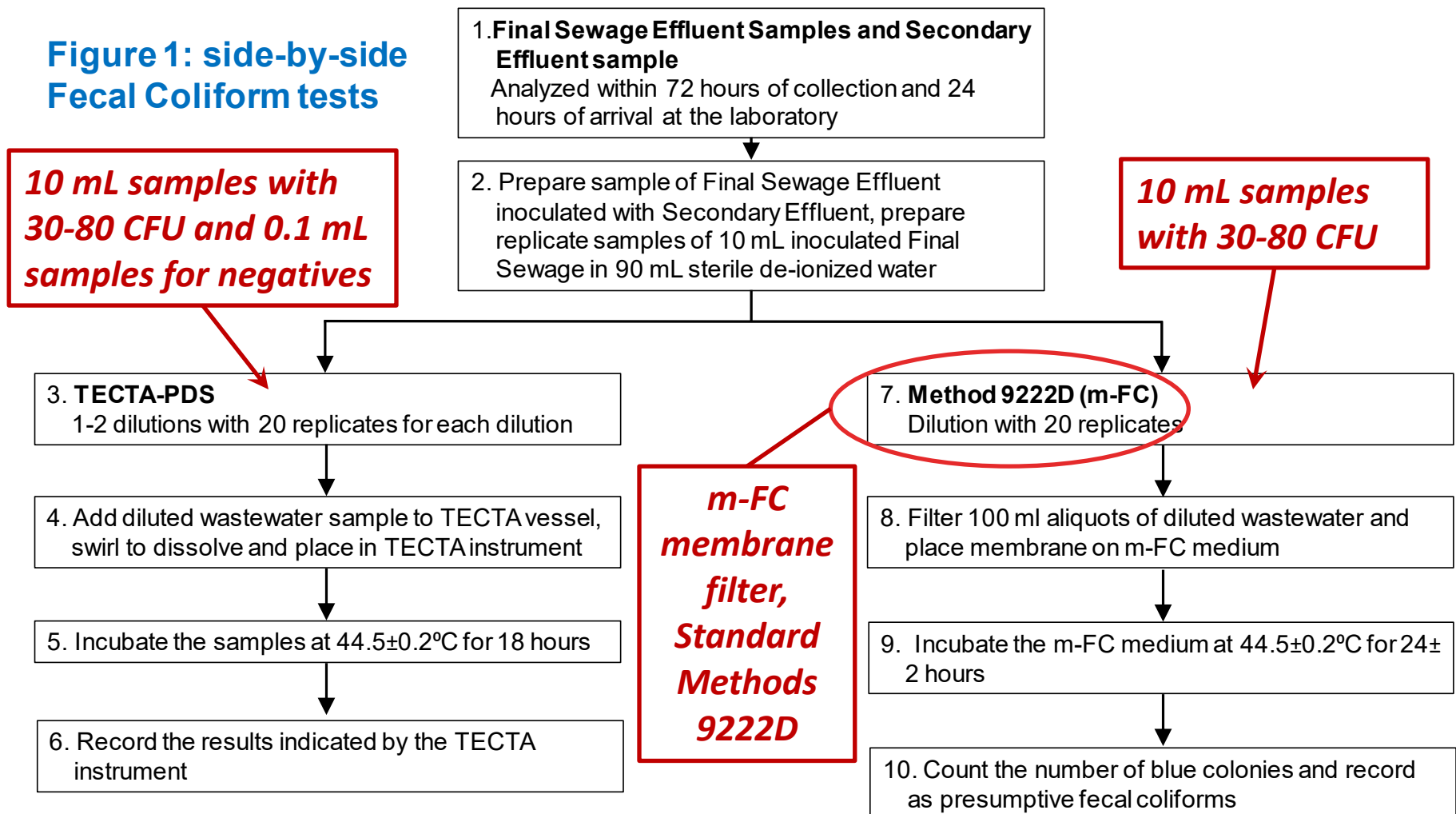
Study Plan – Fecal Coliform Flow Chart

Figure 1: side-by-side Fecal Coliform tests



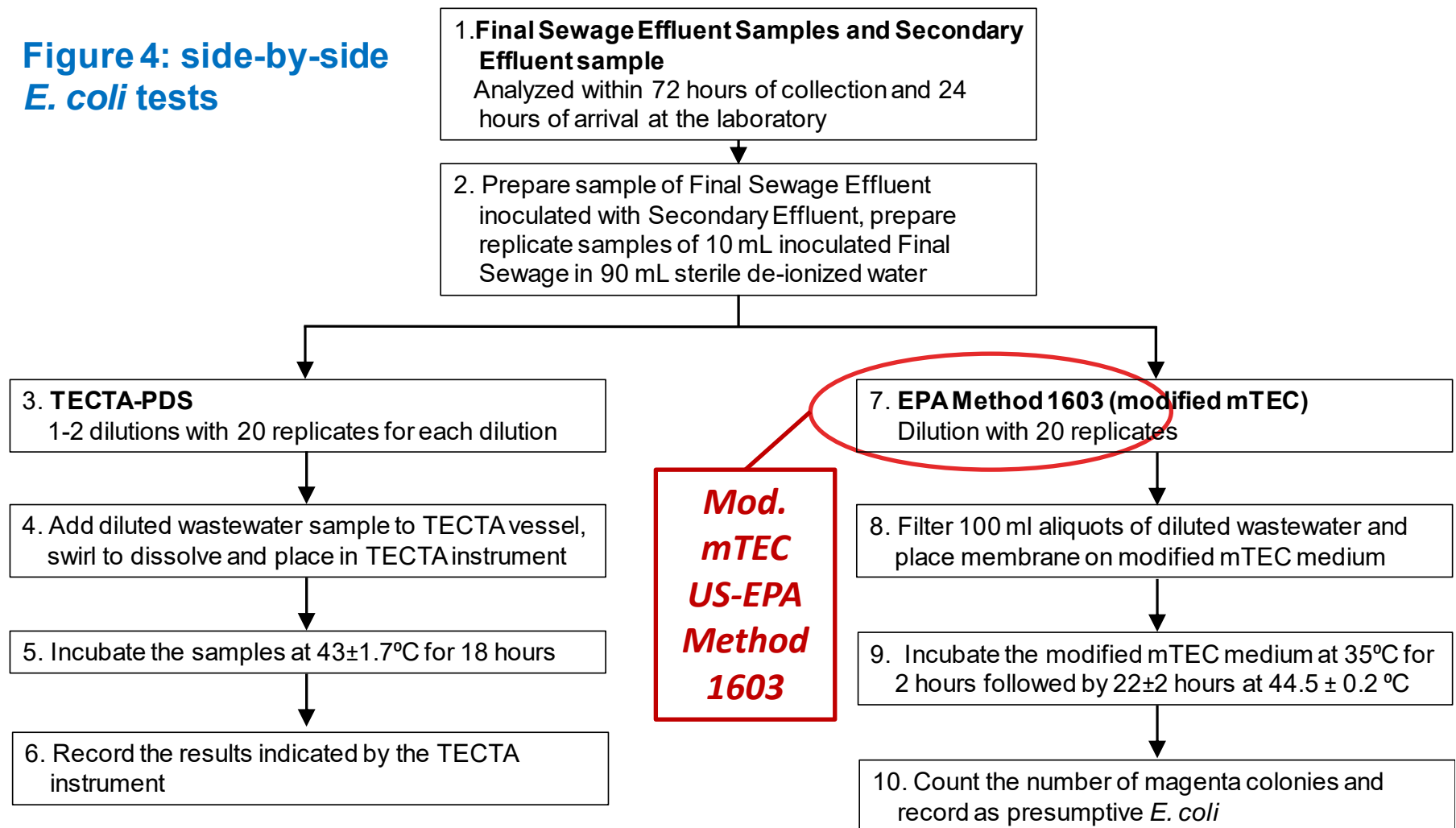
Study Plan – Fecal Coliform Flow Chart

Figure 1: side-by-side Fecal Coliform tests



Study Plan – *E. coli* Flow Chart

Figure 4: side-by-side
E. coli tests



Fecal Coliform Confirmation Flow Chart

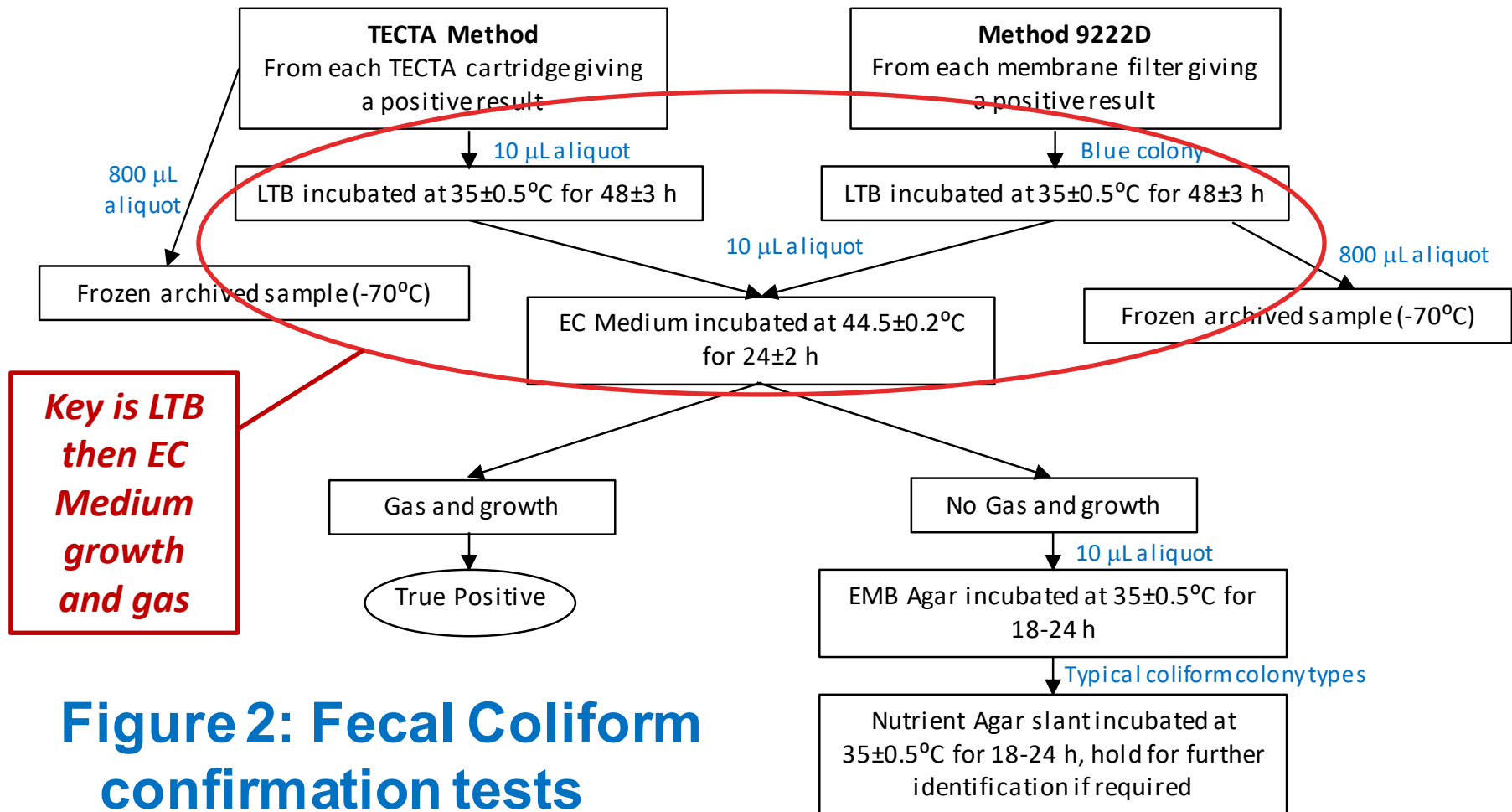


Figure 2: Fecal Coliform confirmation tests

E. coli Confirmation Flow Chart

Isolates from positive samples are pos. for β -D-glucuronidase

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

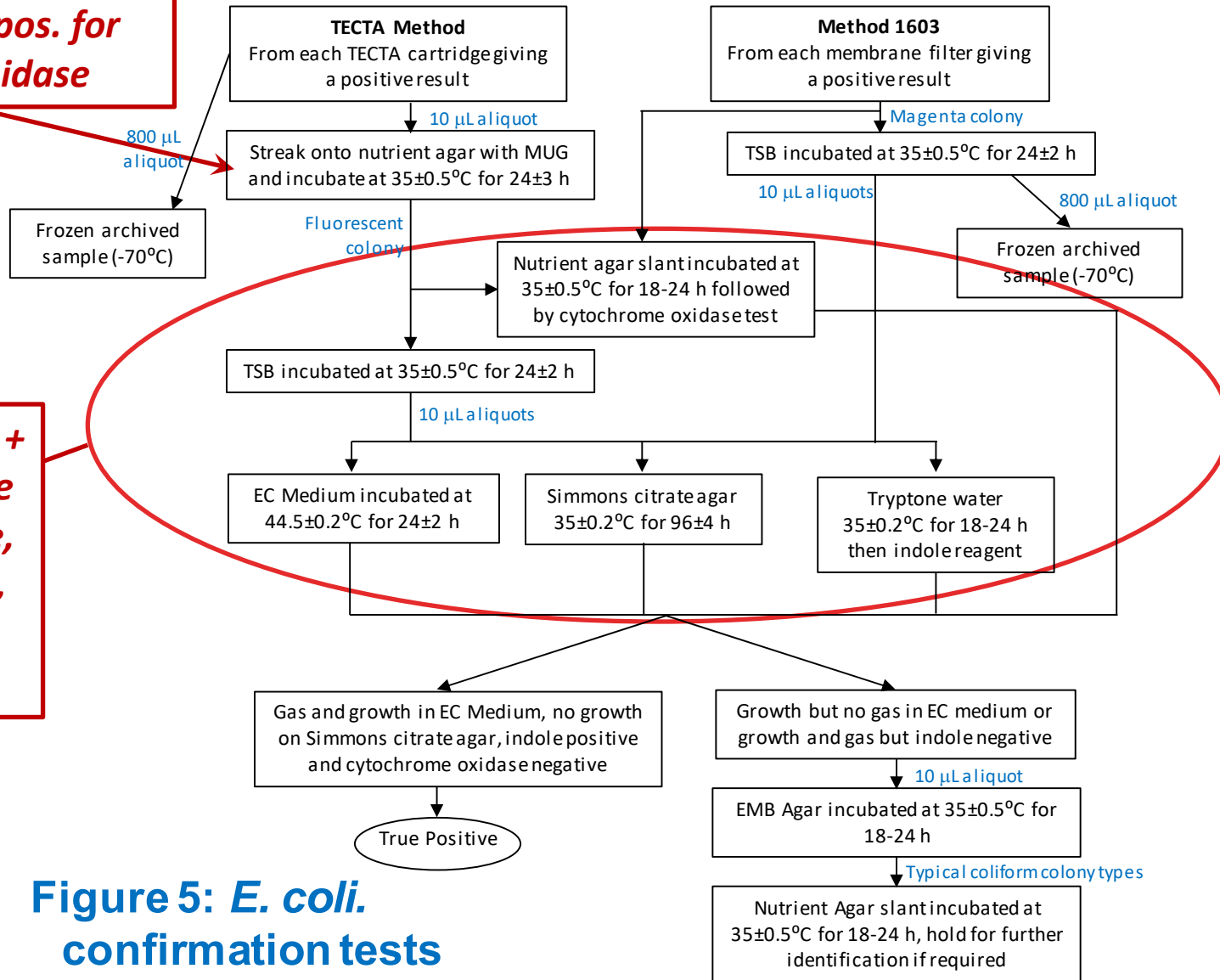


Figure 5: *E. coli* confirmation tests

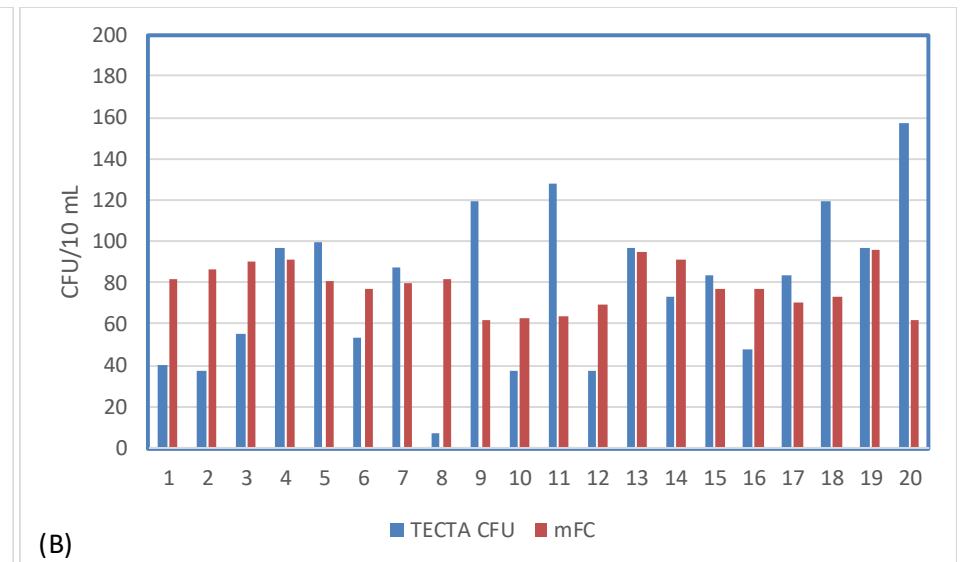
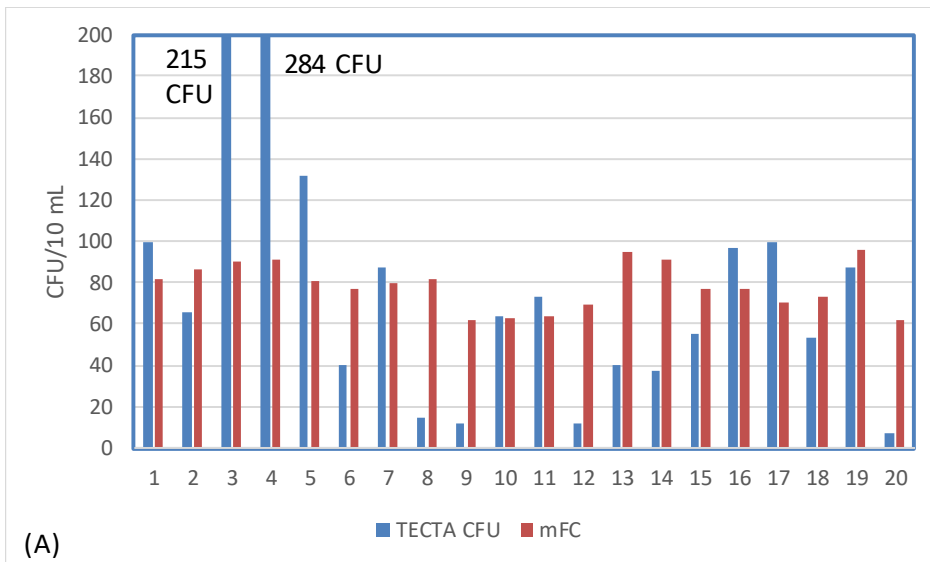
In-House Pre-Study

- In-House Pre-Study done in microbiology lab at Queen's University to validate Study Plan
 - confirm protocol is feasible by 1-2 technicians
 - determine timing and logistics
 - trouble-shooting steps that don't work
 - generate preliminary data to justify full study
- Final and secondary sewage effluent obtained from three WWTPs
 - Final sewage all <10 CFU/10 mL, spiked with secondary
 - Emphasis for reference tests was enumeration (not confirmations)

Note: initial plan in Jan 2020 was to have data from full ATP study and present this in Minneapolis. Hopefully next year...

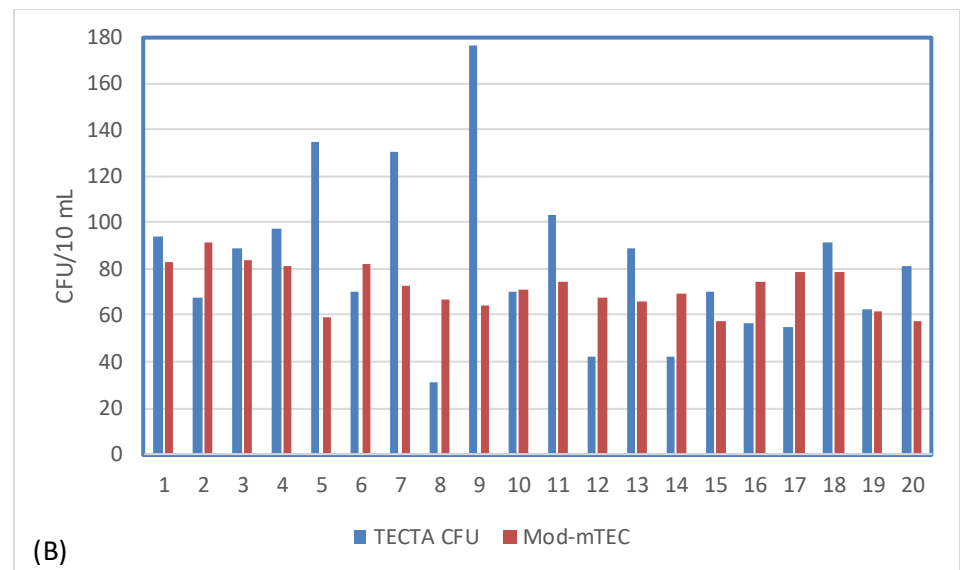
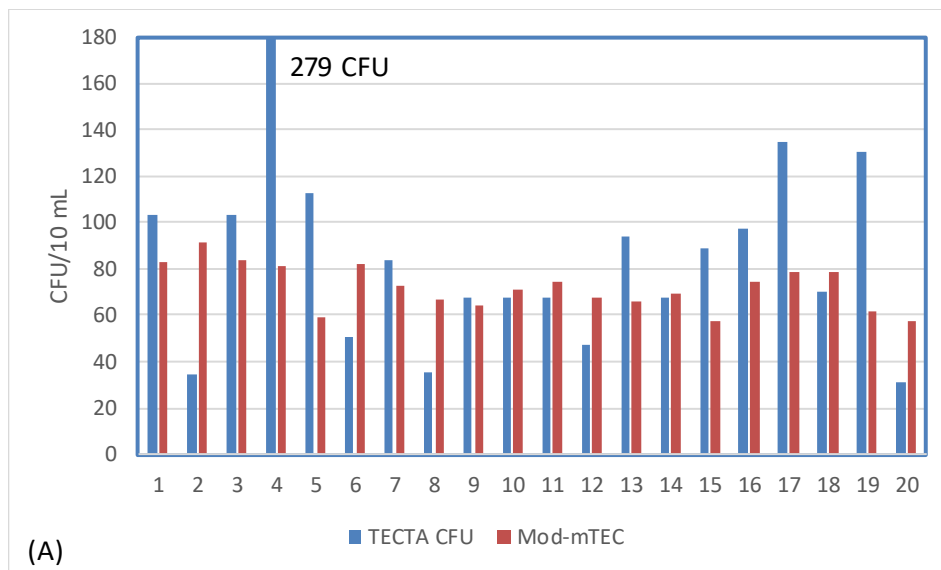
In-House Pre-Study

- Fecal coliform (FC) quantitation
 - results statistically identical between TECTA and mFC



In-House Pre-Study

- *E. coli* (EC) quantitation
 - results statistically identical between TECTA and mFC



In-House Pre-Study

- Confirmation results for 120 TECTA samples
 - All parameters within expected range (>90%)
 - Need more negative samples to balance results

Concordance Rate

$$= (TP+TN)/(TP+FP+TN+FN) \times 100\%$$

False Positive Rate

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

False Negative Rate

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

Sensitivity

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

Specificity

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

Result	EC results	FC results
TP	70	78
FP	4	0
TN	46	41
FN	0	1
Concordance Rate	96.7%	99.2%
FP Rate	8.0%	0.0%
FN Rate	0.0%	1.3%
Sensitivity	100.0%	98.7%
Specificity	92.0%	100.0%

Summary

- Draft ATP Study Plan validated
 - protocol is feasible by 1 technician, faster if some steps done by 2 technicians
 - overall 12-16 weeks expected for full study
 - some refinement of TECTA confirmations needed
- Overall performance of TECTA system supports full Study
 - Quantitative performance matches reference methods
 - Confirmation tests confirm sensitivity and selectivity over 90% for both tests

Acknowledgements

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Questions?

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