

Assessing a New Bacteria Monitoring Technology by Using the US-EPA ATP Protocol to Compare with Reference Methods

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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



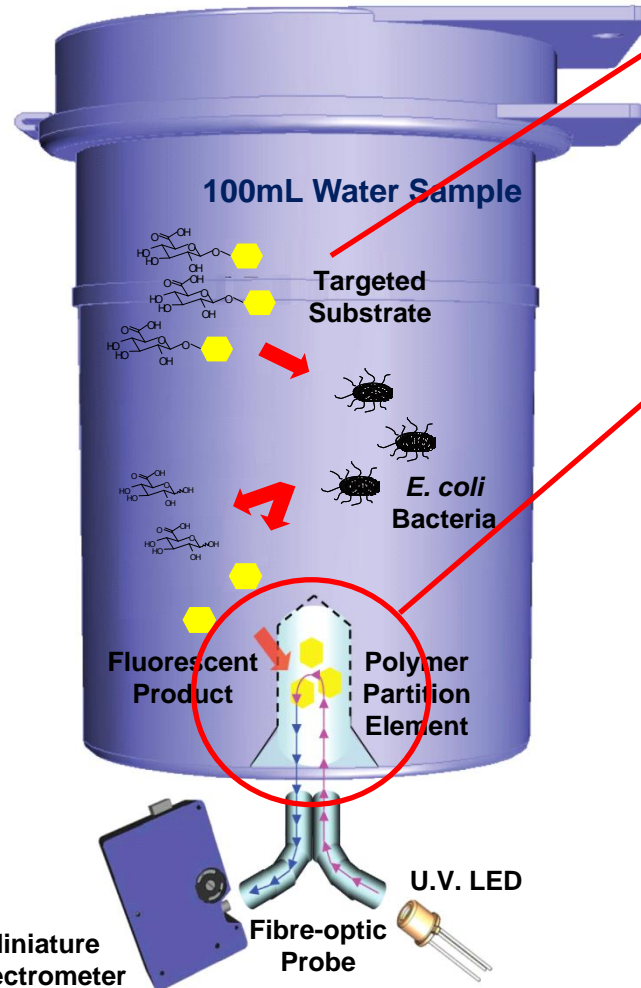
Preloaded, single use
test cartridges

Method overview

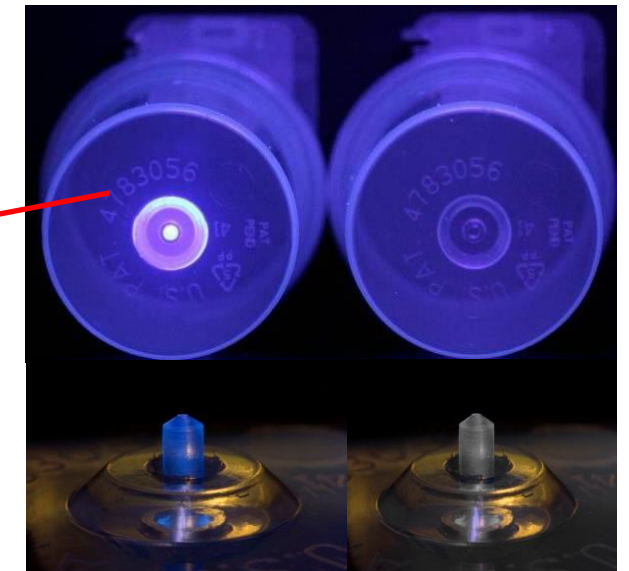
Enzyme-substrate / solution culture method

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

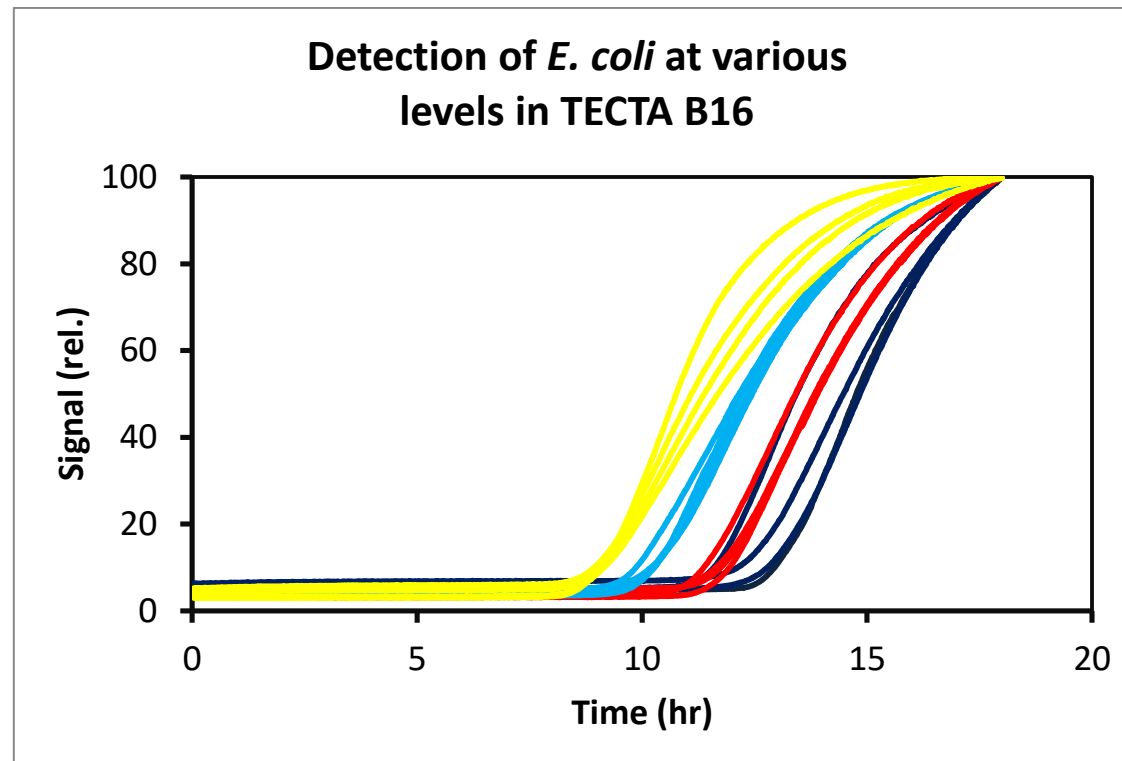


Automated detection of fluorescence
in polymer triggers result



Signal Monitoring and Quantitative Analysis

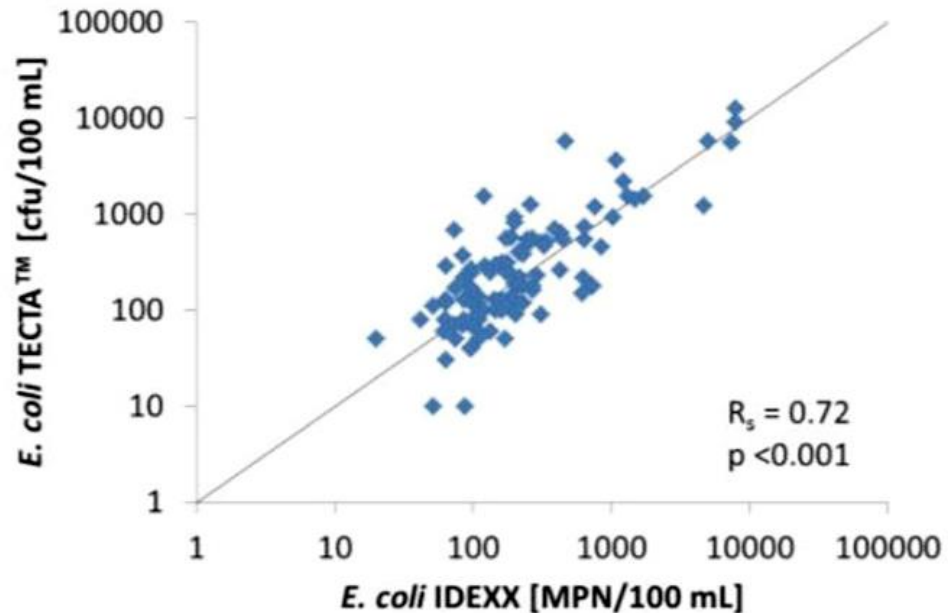
- Signal monitored constantly from polymer in cartridge
 - growth and enzyme expression produce “growth curve”
 - signal onset gives Time-to-Detection (TTD)
 - TTD linearly related to log initial bacteria level



Validation of Quantitative Analysis

- 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.

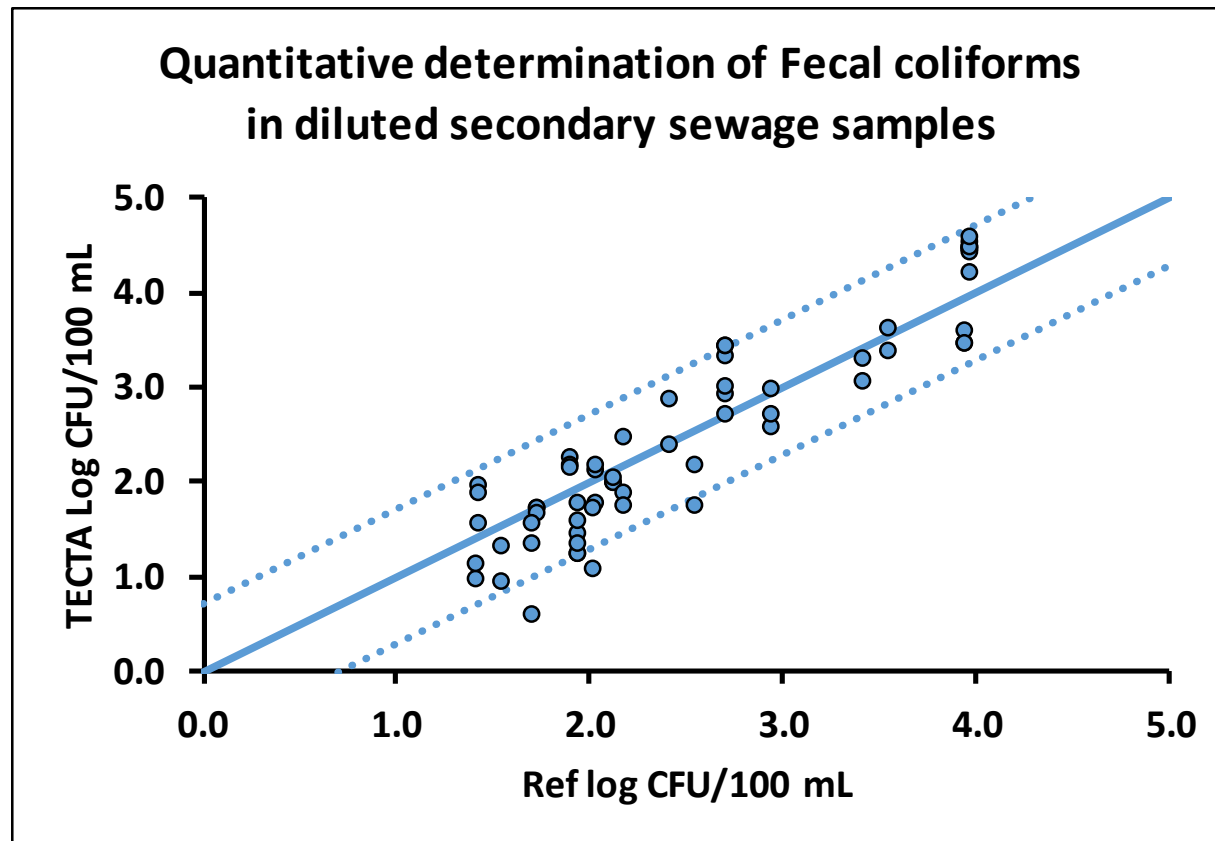


- Similar results reported by Prevost group, U. de Montreal

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.

Quantitative Test for Fecal Coliforms

- Validation of default calibration from previous data
 - FC is Total Coliforms test run at 44.5 °C
 - Performance similar to Total Coliforms test



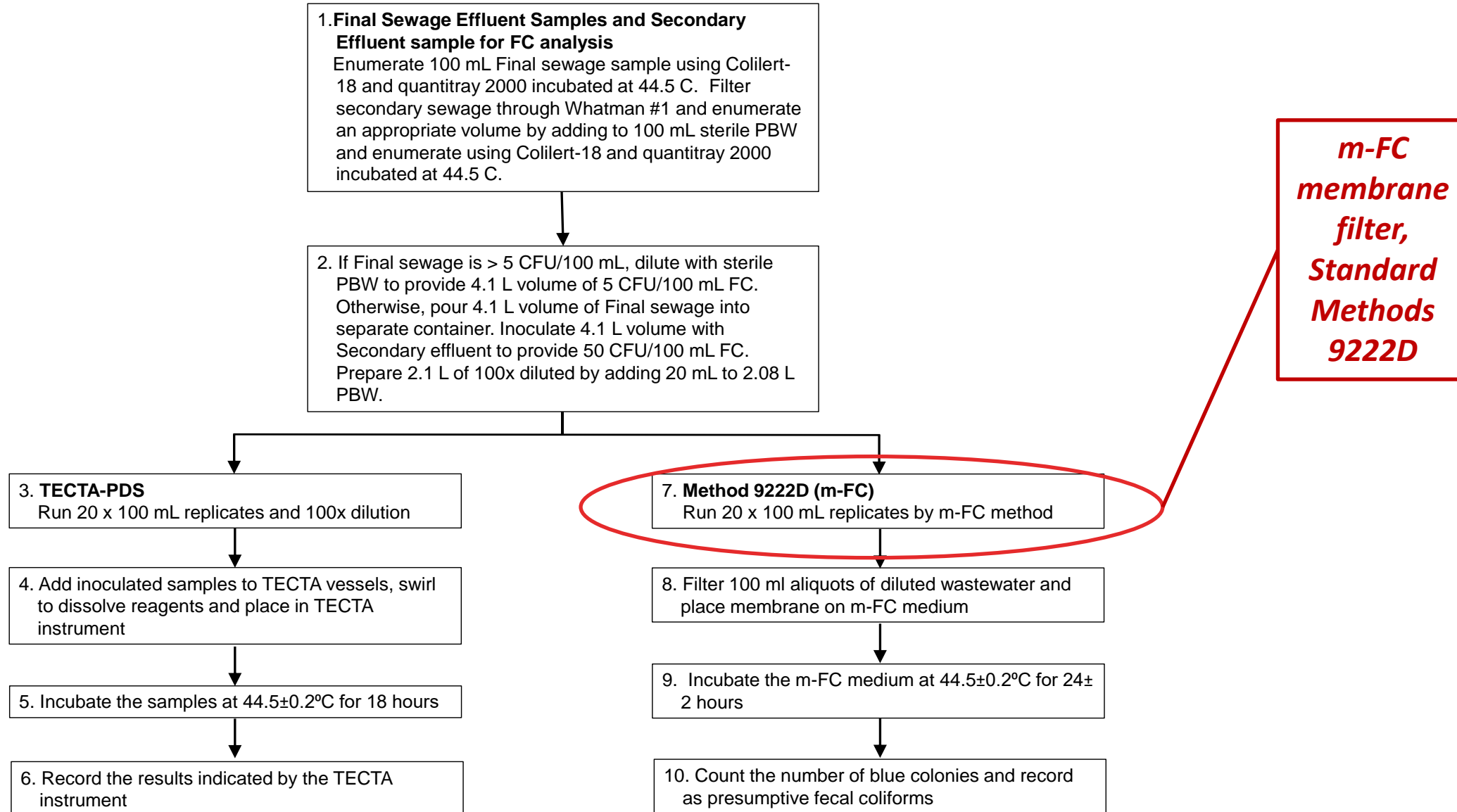
Goal: EPA approval of method for wastewater

- 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 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 (fecal coliforms) and modified m-TEC (*E. coli*)

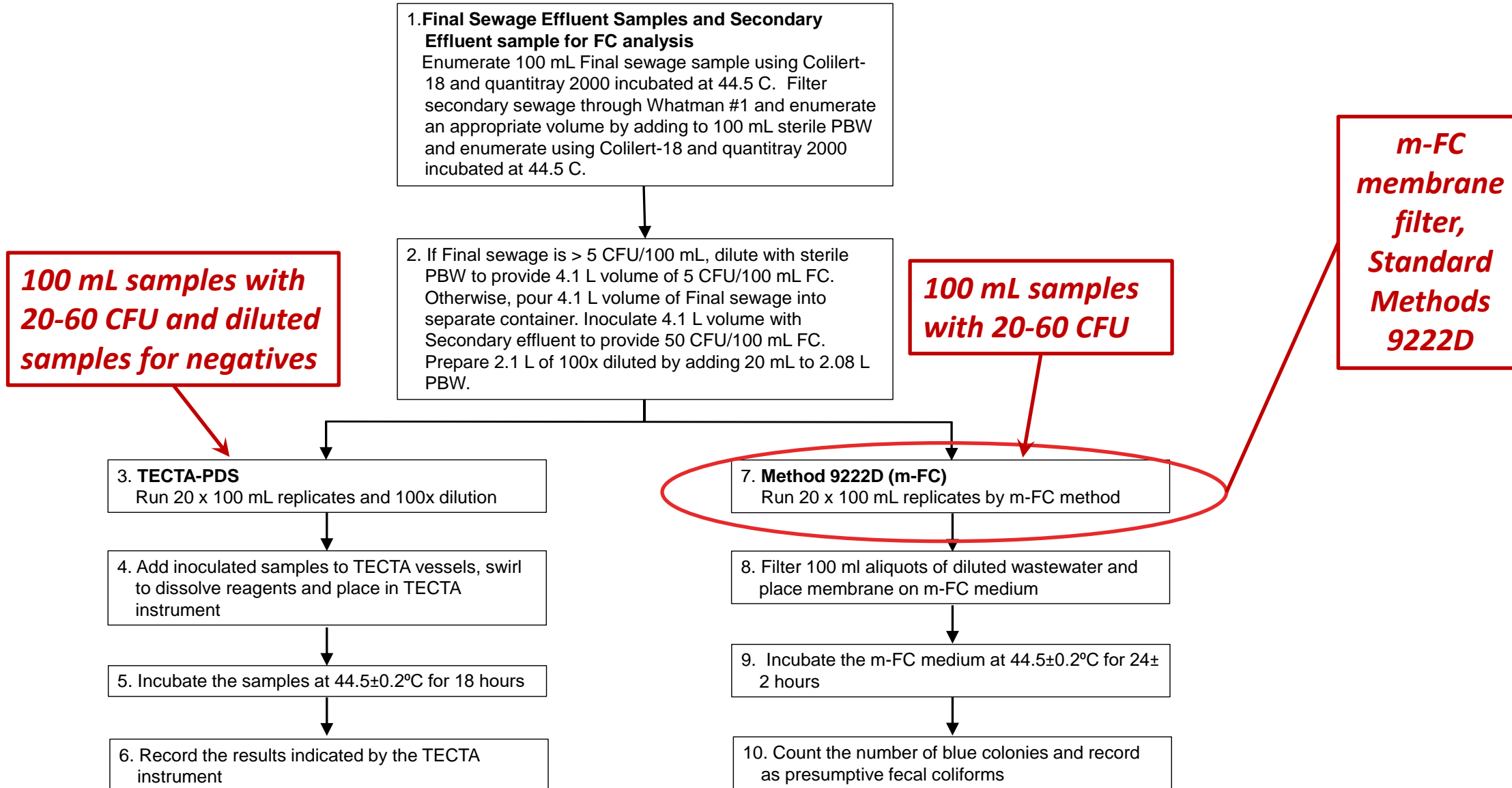
Study Plan details

- 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 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

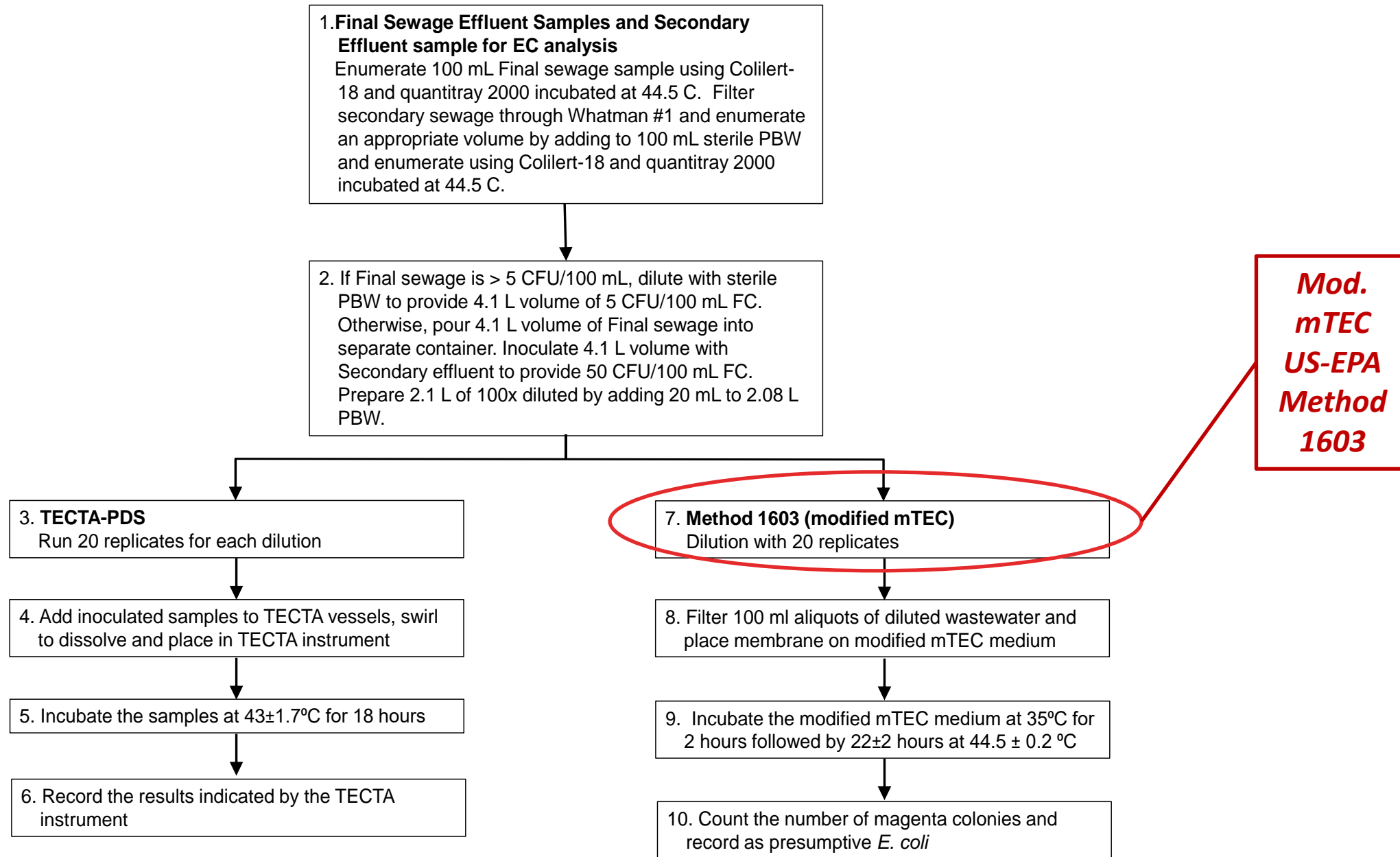
Study Plan – Fecal Coliform Flow Chart



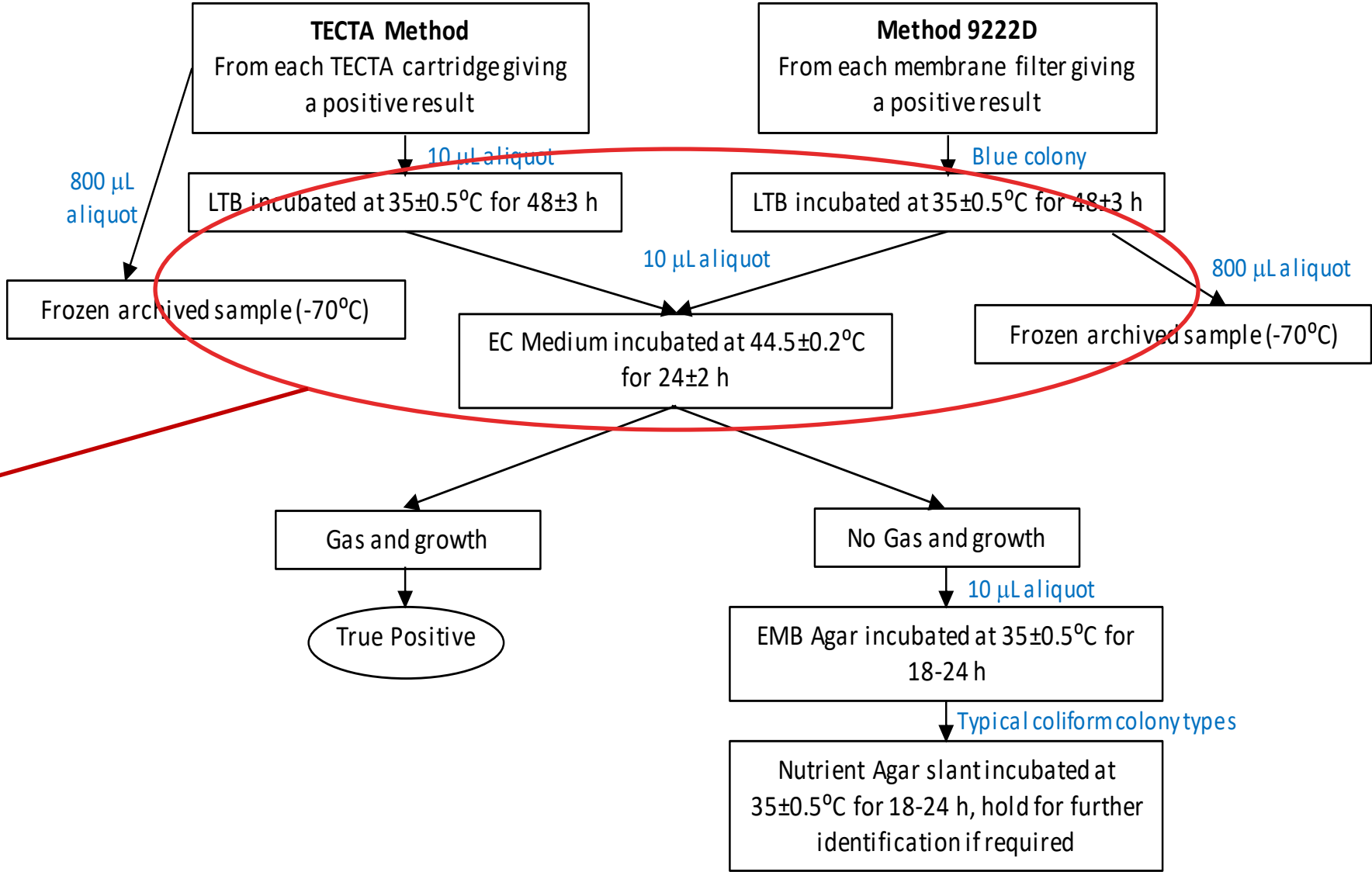
Study Plan – Fecal Coliform Flow Chart



Study Plan – *E. coli* Flow Chart



Fecal Coliform Confirmation Flow Chart

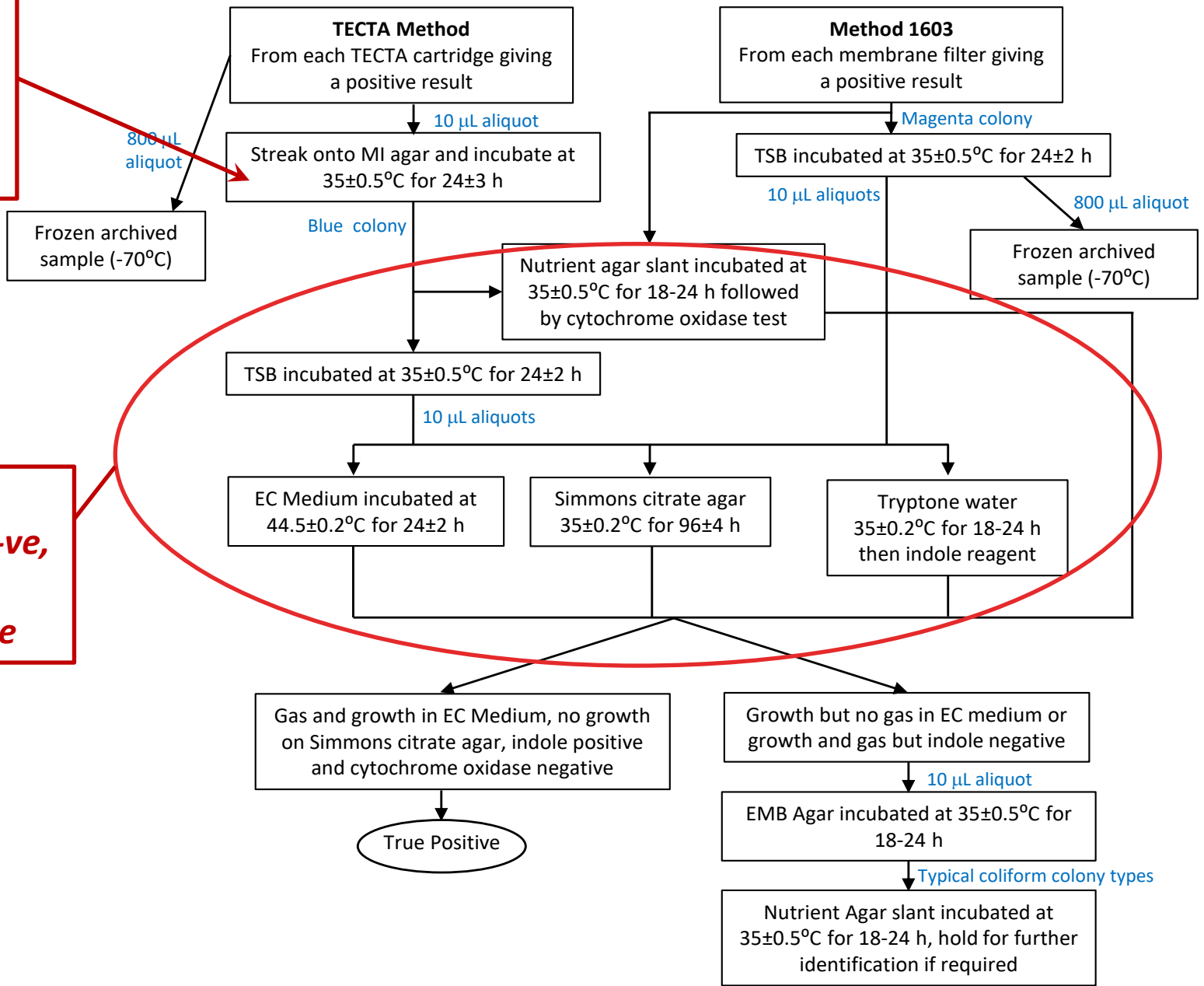


**Key is LTB
then EC
Medium
growth
and gas**

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



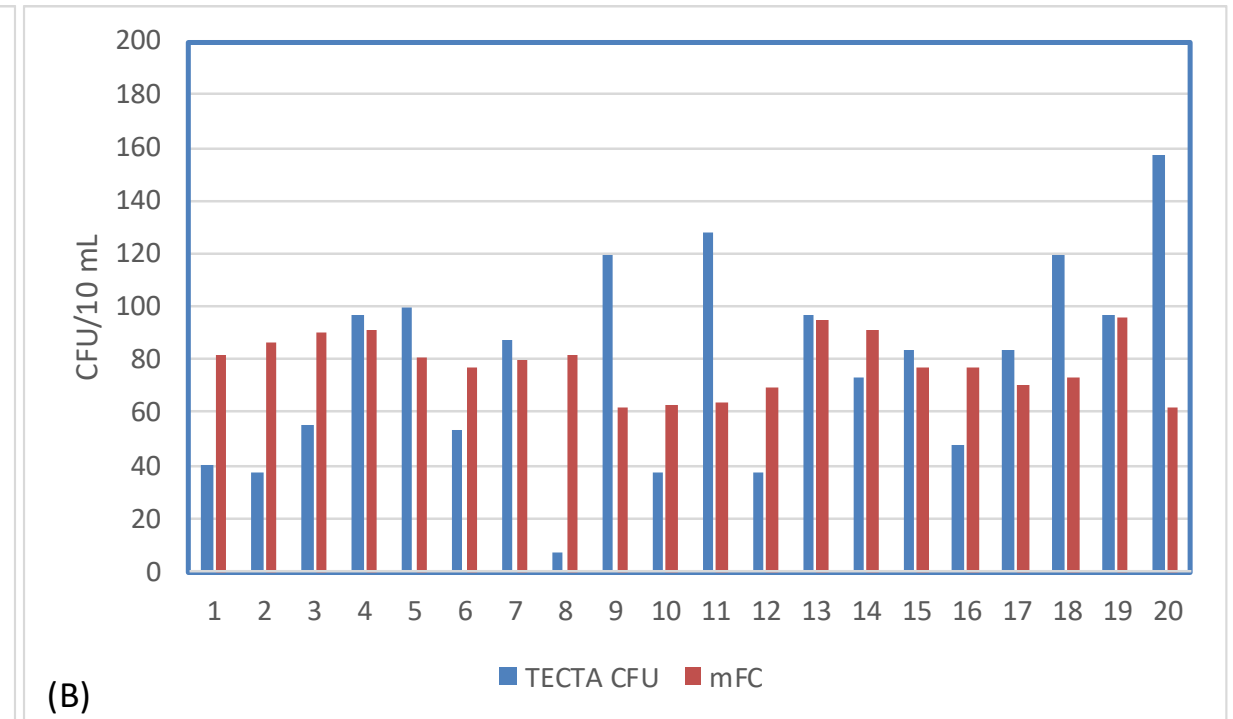
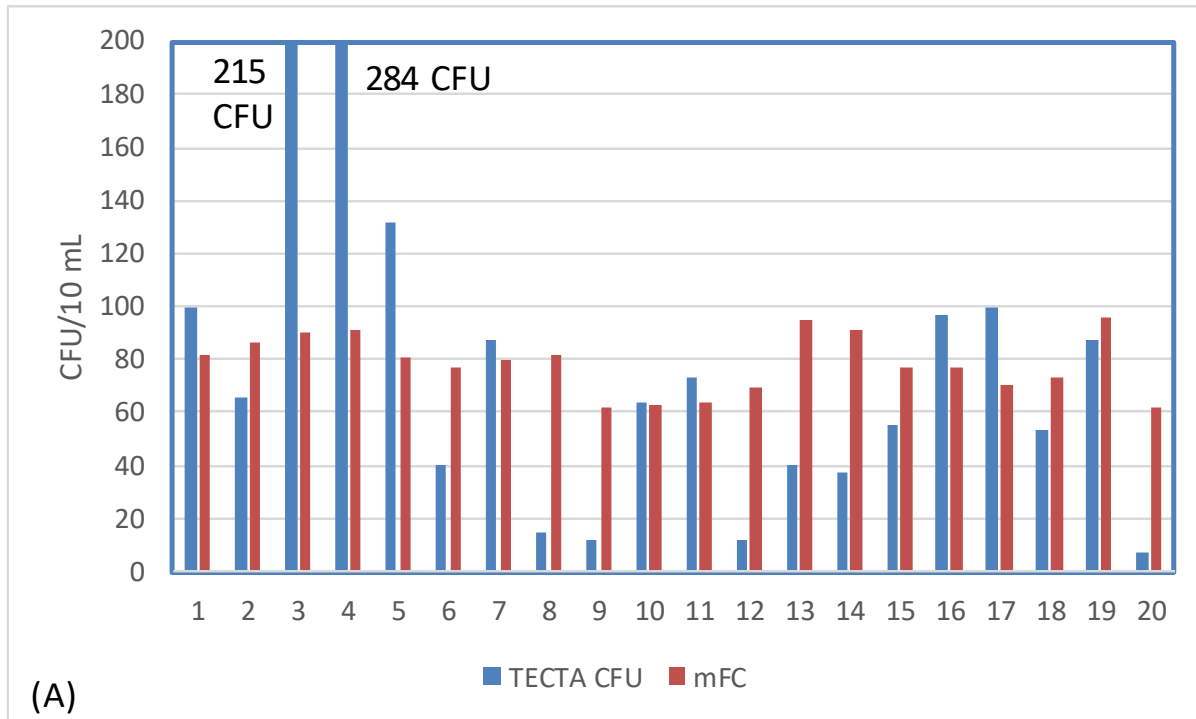
In-House Pre-Study

- 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 <5 CFU/100 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 Aug 2020.
Now plan for final data in 2022...*

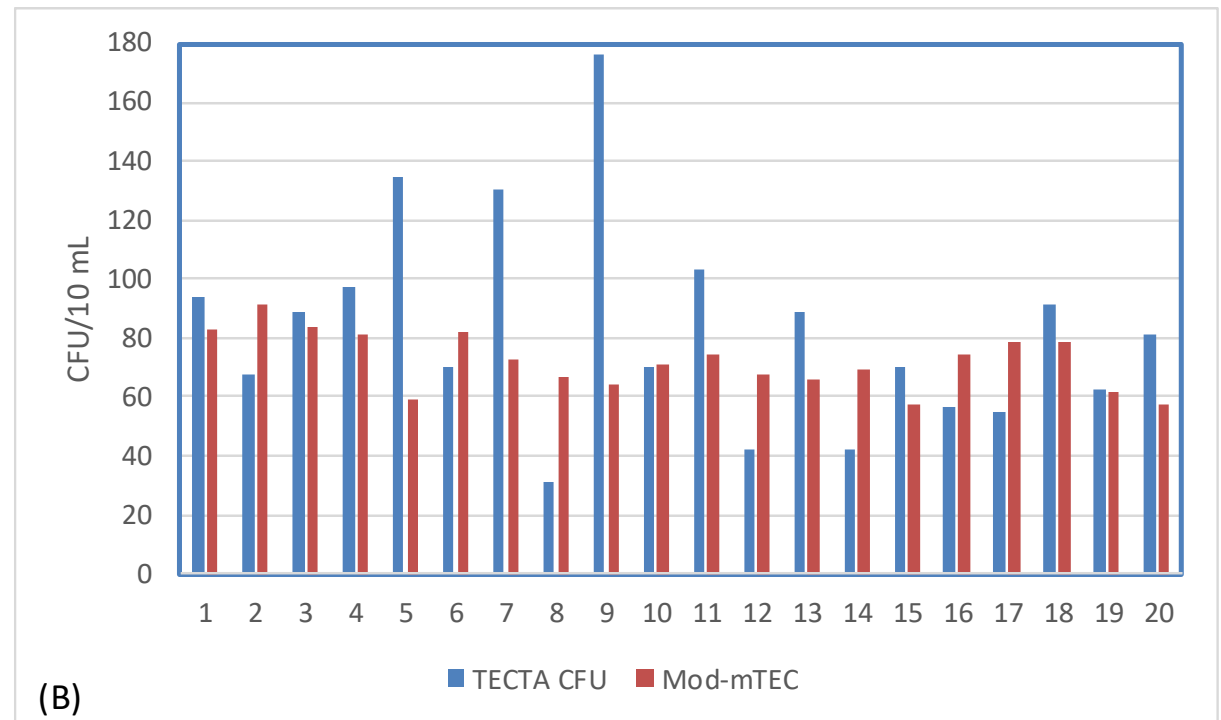
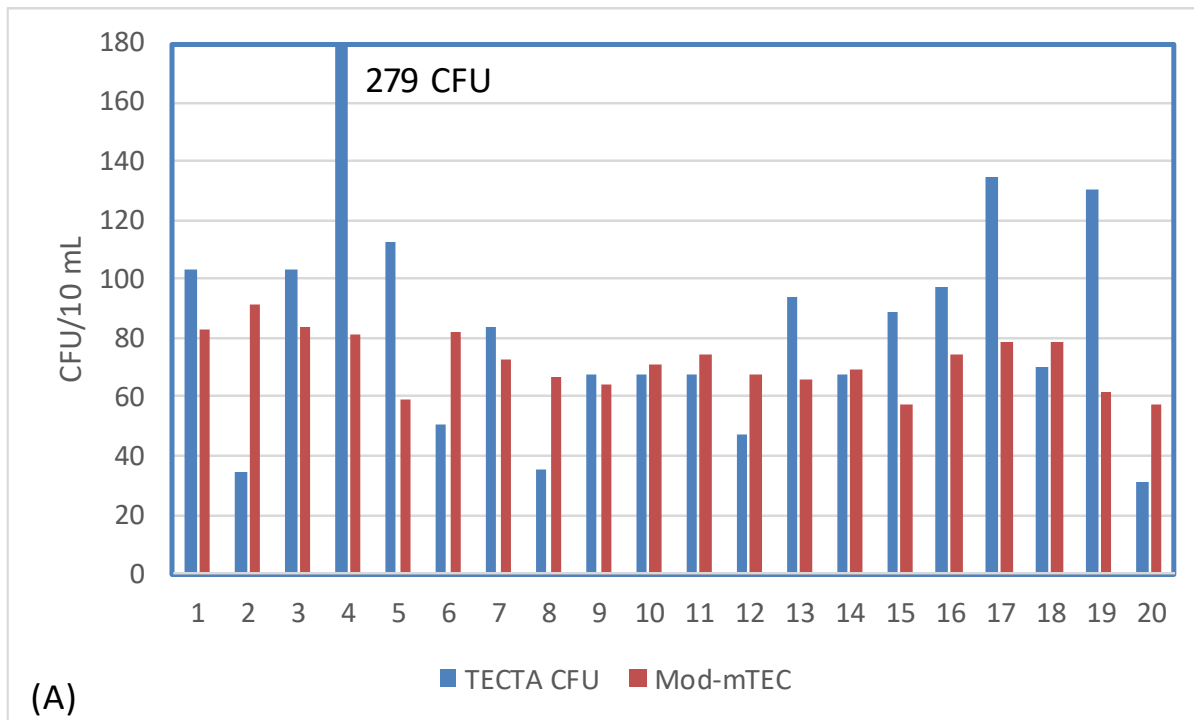
In-House Pre-Study

- Fecal coliform (FC) quantitation
 - all positive TECTA samples detected in under 10.6 hours
 - results statistically identical between TECTA and mFC ($p > 0.05$)



In-House Pre-Study

- *E. coli* (EC) quantitation
 - all positive TECTA samples detected in under 9.4 hours
 - results statistically identical between TECTA and modified mTEC ($p>0.05$)



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 TECTA system performance supports going ahead with 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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