Microbiology Method Variability and Sensitivity in the Detection of Total Coliforms and *E.coli*

Shawn Kassner and Curtis Wood

ERA

A Waters Company

Agenda

- Introduction
- Background
- Procedure for data review
- Results
  - Qualitative Data Review
  - Quantitative Data Review
- Literature Review
- Conclusions/Recommendations

Introduction

- Roles of proficiency testing per ISO 17043 *Conformity assessment – General Requirements for Proficiency Testing*
  - "evaluation of the performance of laboratories"
  - "establishment of the effectiveness and comparability of test or measurement methods"
- Through the periodic review of proficiency testing data, trends emerge as to the performance of methods and technologies

Background

- ERA has received various requests over the last several years for technical assistance from laboratories with “Not Acceptable” results.

- These requests led ERA to see trends in the recoveries and failure rates of various methods for microbiology.

- July 2010 Total Coliform Rule, 40 CFR 141 and 142, volume 75 Number 134
  - “EPA is also aware of reports of varying performance of some enzyme substrate based methods.”
  - EPA would like to have all of the drinking water methods to be evaluated through the Environmental Technology Verification Program (ETV).

Background

- Through the ETV process “EPA would judge the appropriateness of each analytical method and would determine which should continue to be approved for future monitoring.”
- EPA proposed dropping Fecal Coliforms as a analyte of concern.
- A stakeholder meeting was held concerning evaluating methods via the ETV process. Outcome of the meeting was that there are budgetary constraints to moving forward with this process.
- Still remains a concern with EPA and they are still very interested in moving forward with the evaluation of the methods.

Procedure

- ERA conducts 18 qualitative and 12 quantitative single blind proficiency testing studies annually.
- Approximately 4250 laboratories participate in the qualitative studies & 2410 laboratories participate in the quantitative studies
- Qualitative studies are 10 sample sets analyzed for presence/absence of Total and Fecal coliforms and *E.coli.*
- Laboratories analyze the samples via their normal procedures and return the data to ERA for evaluation.

Procedure

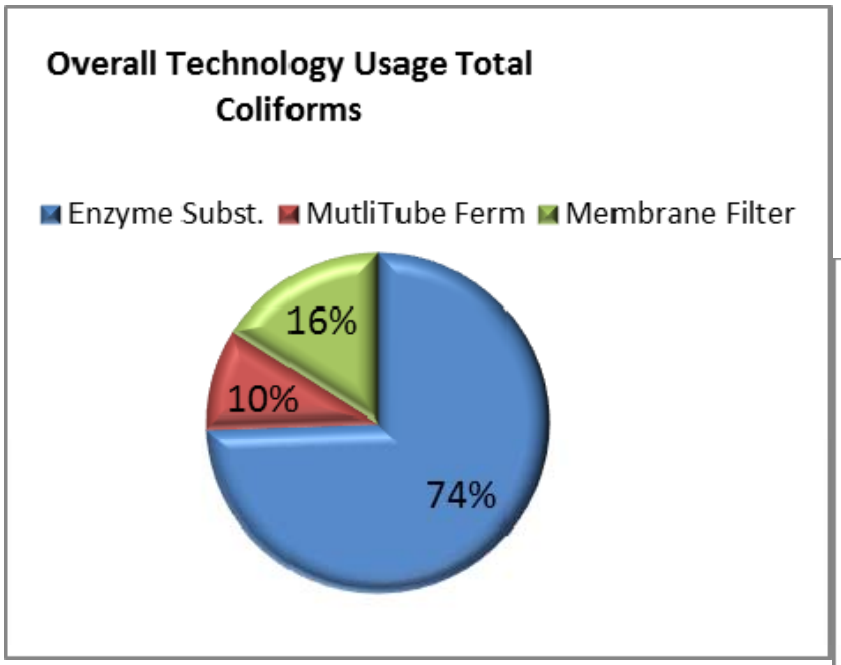
- ERA collated the data from 26 proficiency testing studies for Total coliforms and E.coli for the qualitative analysis

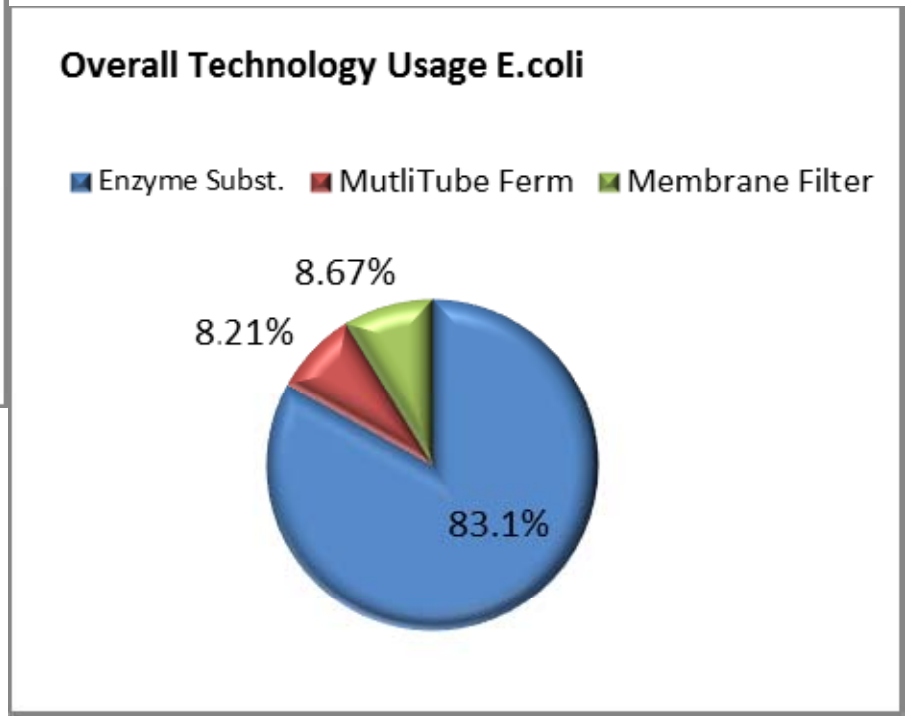
- ERA collated the data from 23 proficiency testing studies for Total coliforms and E.coli for the quanitative analysis

- The data were broken into the categories
  - General analytical types
    - Membrane filtration, multiple tube fermentation, enzyme substrate
  - General analytical methods and manufacturer
    - SM9222, SM9223, SM9221
    - Colilert™, Colilert-18 ™, Colisure ™, mColiBlue ™, etc.

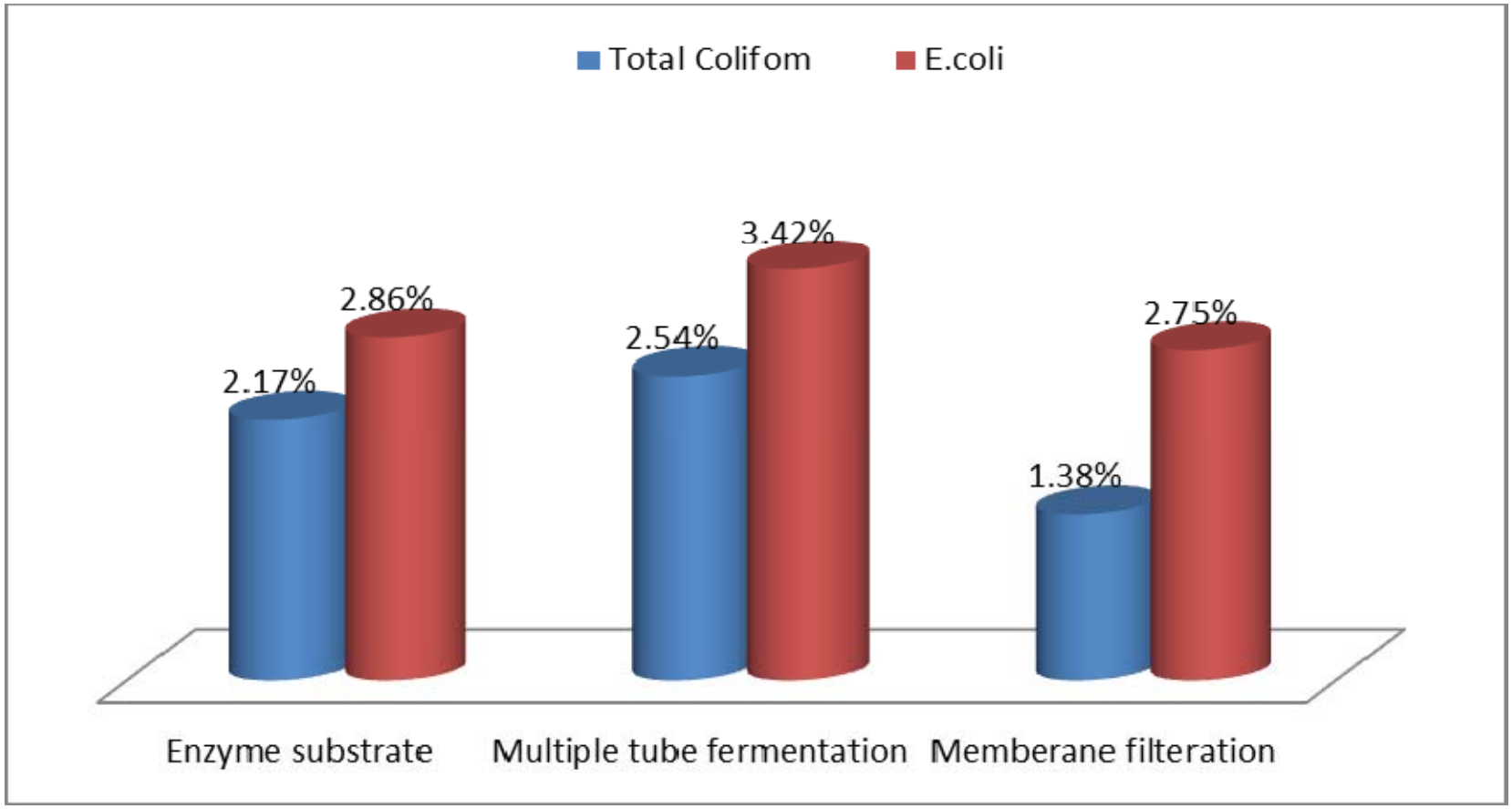
Qualitative Data Usage

- Method Usage by Technology

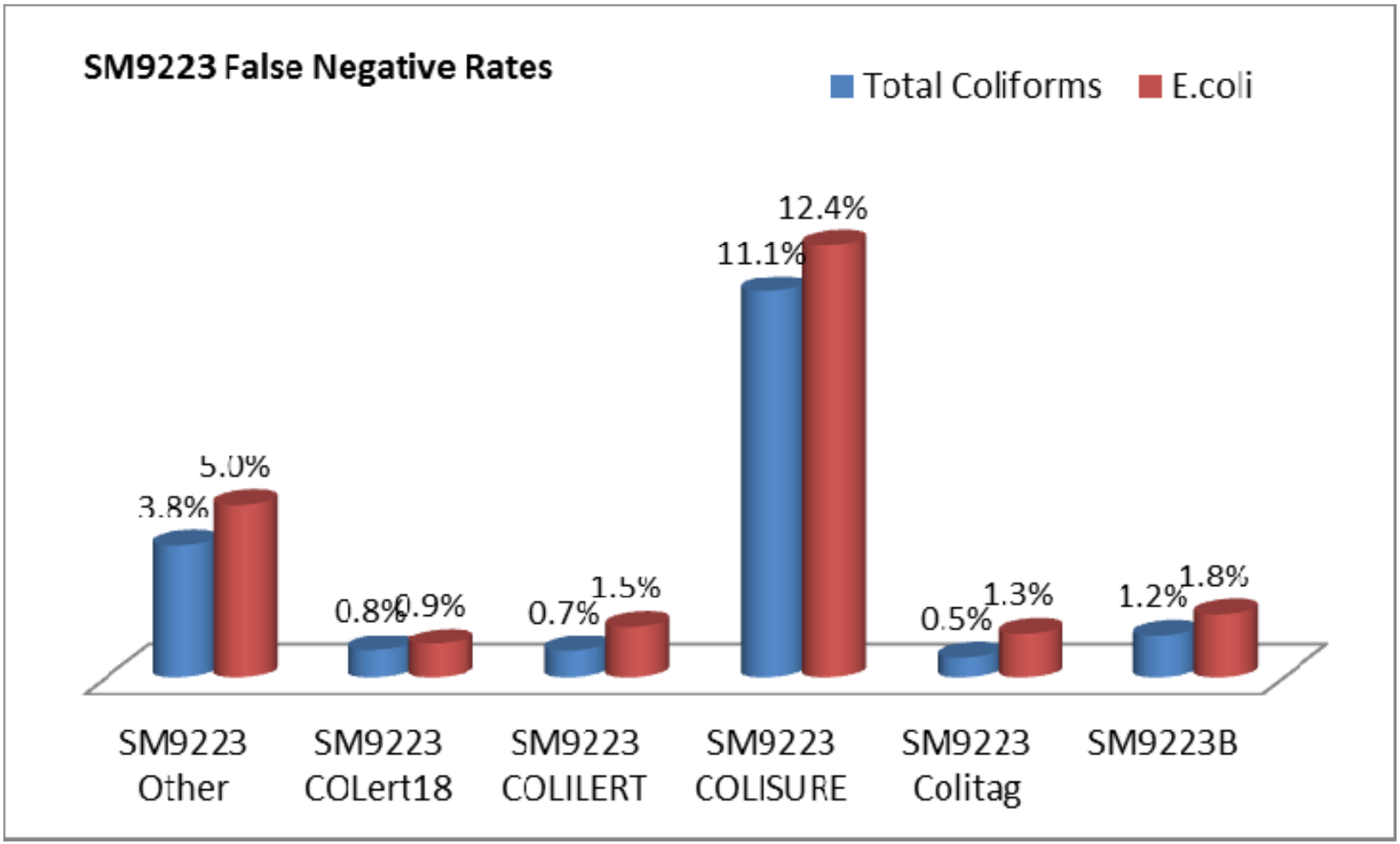




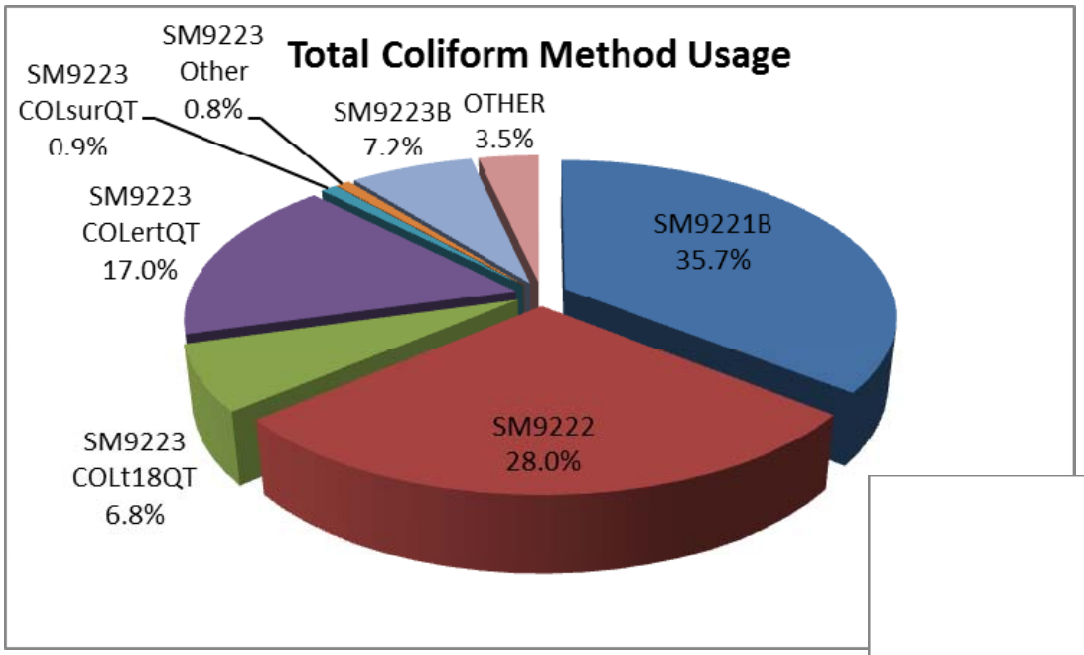
Overview of Technology Data False Negative Rates

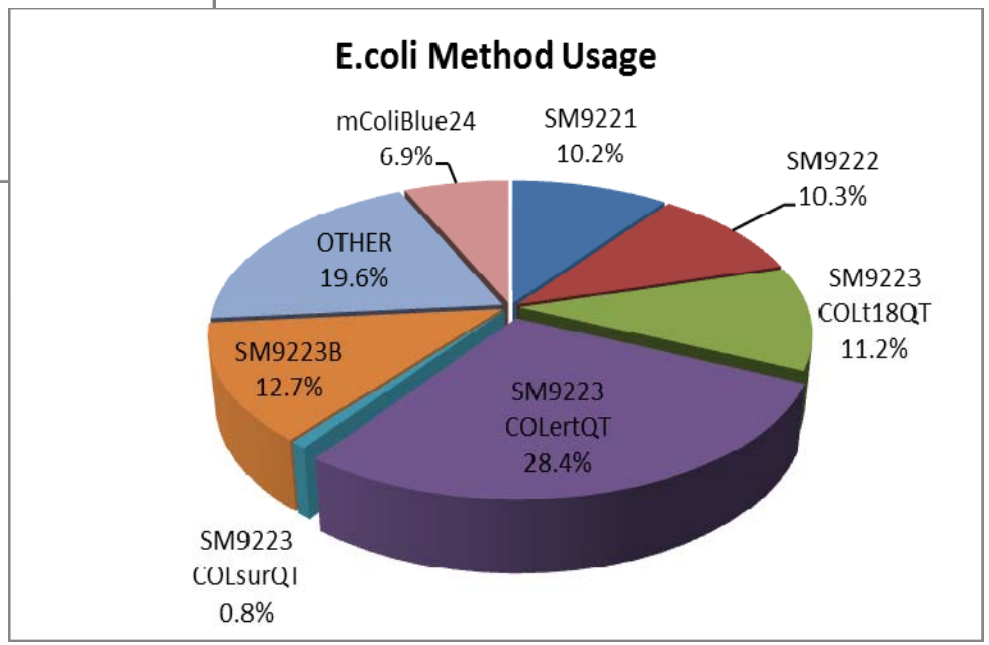


Enzyme Substrate Method
False Negative Rates

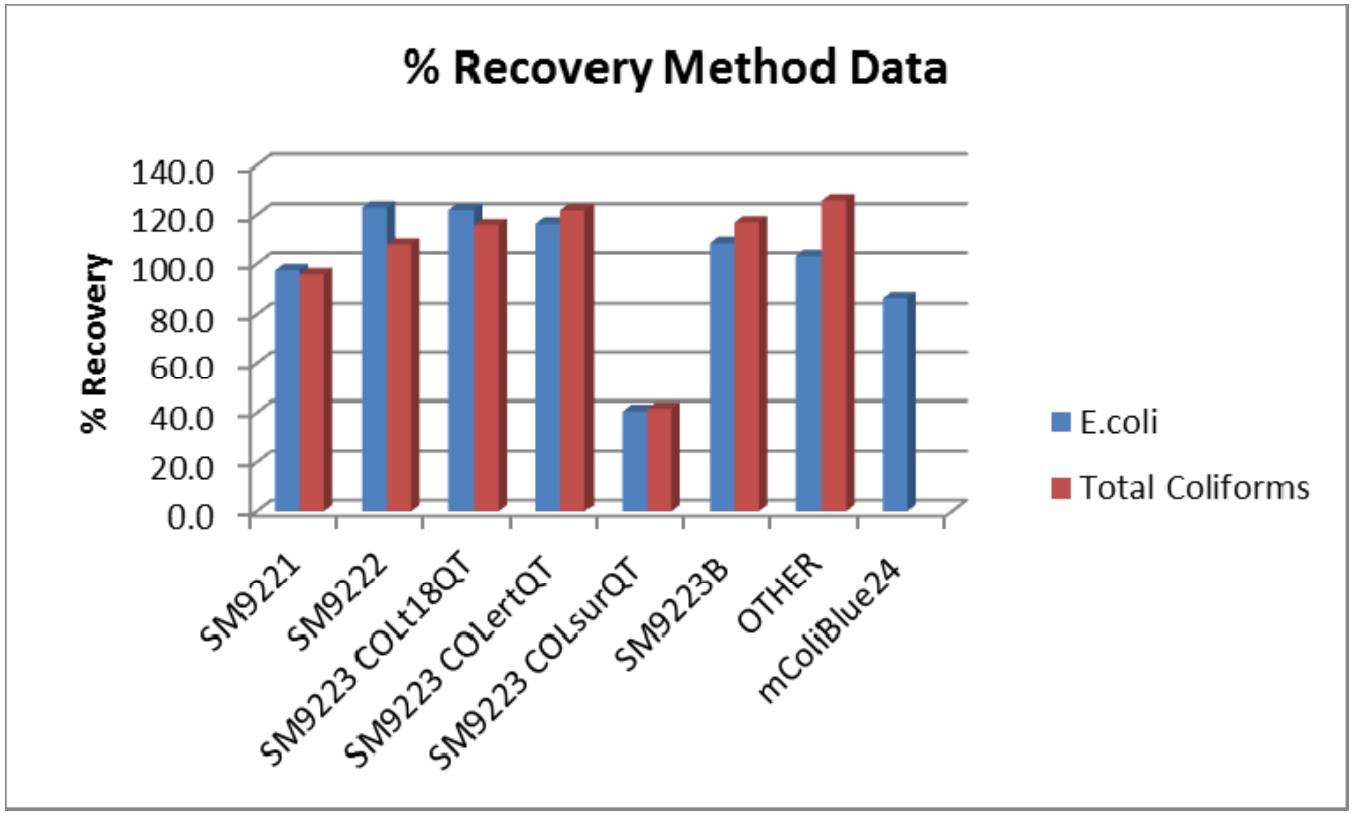


Quantitative Method Usage Data

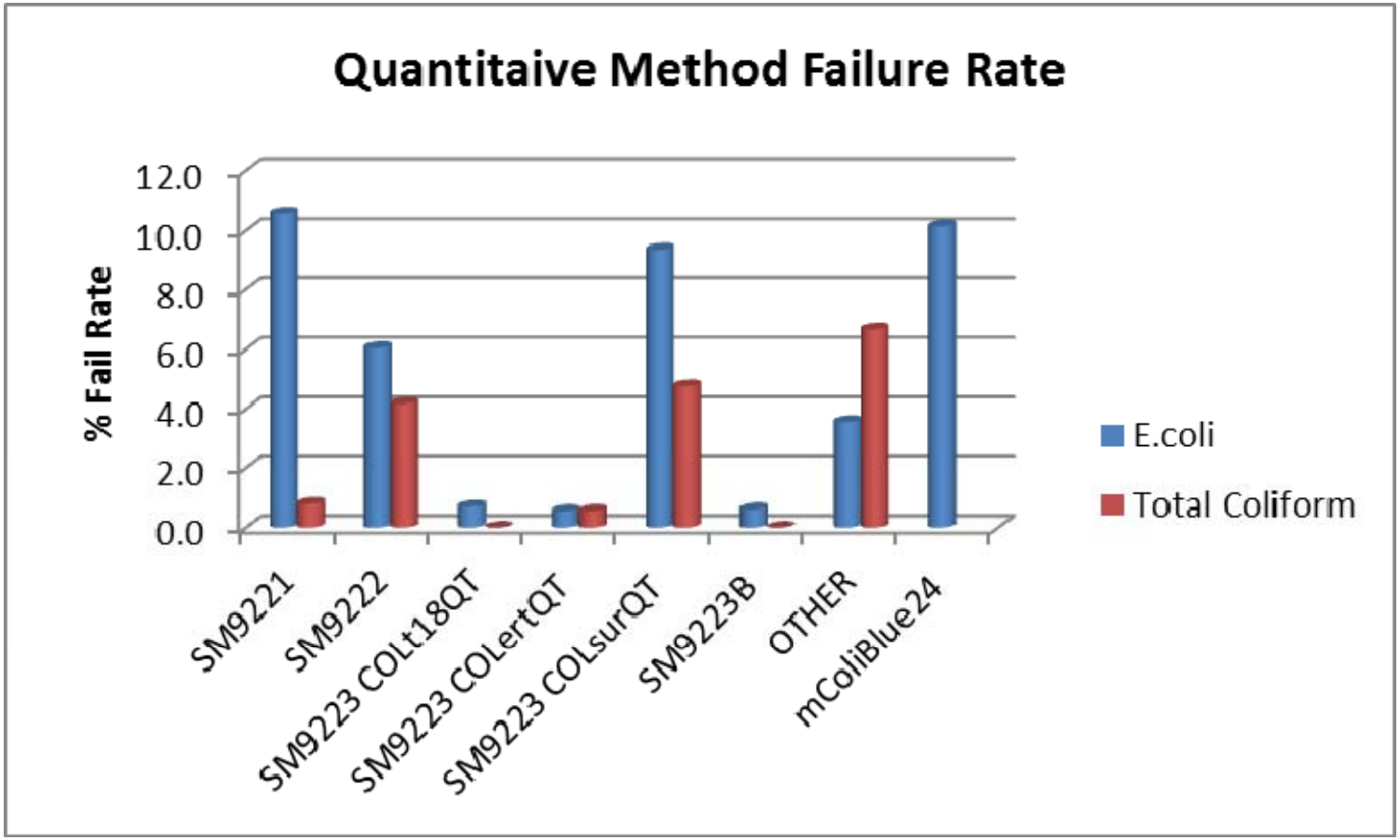




Quantitative Mean Recovery by Method



Quantitative Fail Rate by Method



Literature Review

- 2007 Wisconsin State Laboratory of Hygiene by J. Olstadt et al
  - Ten EPA approved drinking water methods for their ability to identify total coliforms and *E.coli* for both qualitative and quantitative analyses for well water.
  - All of the methods were enzyme substrate methods, where one method MI Agar® used in conjunction with membrane filtration.
  - The methods were used to detect five different organisms at two different concentration ranges. (< 10 and 50 – 100)
  - Organisms used were isolated from the drinking water samples collected by the Wisconsin State Laboratory of Hygiene
  - Matrix selected was from three different ground Wisconsin water sources

Literature Review

- 2007 Wisconsin State Laboratory of Hygiene by J. Olstadt et al

  - Each method was analyzed in triplicate. The method was considered a false negative when the all three reported results were recorded as ‘absent’ for the presence/absence testing.

  - A false negative failure rate of 0% for Colilert, 3.3% for Colilert-18, 0% for Colisure-48, for 20% for Colisure-24, and 23% for mColiBlue-24 for presence/absence testing.

  - Colisure-24 and mColiBlue -24 had a lower average percent recovery across the three different sites for both concentration ranges tested when compared to the other eight methods tested.

Literature Review

- "Evaluation of the methods for enumerating coliform bacteria from water samples using precise reference standards" by T.Wohlsen et al, in 2005.
  - Tested eight different methods for their ability to accurately measure concentrations of *E.coli* and *Enterobacter aerogenes* at a concentration of 30 organisms per 100 mL sample of sterilized tap water.
  - The organisms used in the study was from BioBall™ cultures.
    - BioBall™ cultures are freeze dried.
  - *E.coli*, membrane filtration, spread plate methods and Colisure "recovered significantly lower mean counts than specified."

Conclusions

- The presence of coliform organisms in the country's drinking waters is an important indicator of potential public health problems.
- The analytical methods used for this analysis need to be able to detect various strains of these organisms, at concentrations ranging from low to high, and at varying levels of stress.
- As noted earlier by EPA, enzyme substrate methods are being reported having varying degrees of performance, across sample types, organisms, and concentrations.

TNI Recommendations

- ERA acquired presence/absence PT samples from other US based, accredited PT providers and programs.
- Quantitative analysis on the presence/absence PT samples was performed and found concentrations ranging from ~ 15 MPN/100 mL to ~ 580 MPN/100 mL.
- Most providers have concentrations under 300 CFU/100 mL, in a countable range.
- ERA is recommending that TNI determine a concentration range that approximates real world conditions and then establish a concentration requirement for the manufacture of these PT samples.

Agency Recommendations

- In discussions with laboratories we found many laboratories were not performing a complete root cause analysis and were just running another PT sample.
- Not always from the same provider.
- State, EPA and third-party laboratory accreditation programs should provide additional guidance and training to laboratories about the proper fulfillment of root cause analysis and corrective action procedures.
- Including whether the method overall is performing adequately.

EPA Recommendations

- We also encourage EPA to conduct a re-evaluation of all microbiology methods via the ETV process to ensure accurate and consistent performance among the many methods that are currently approved.

Questions???

Thank you for your time!

Shawn Kassner

skassner@eraqc.com

Curtis Wood

cwood@eraqc.com