Methods of *E. coli* Enumeration: Considerations and Implications

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Clean Water Act – Water Quality Standards

- Clean Water Act – 303(d)
  “restore and maintain the chemical, physical and biological integrity of the Nation’s waters”

- Water Quality Standards

- Fecal Indicator Bacteria – *E. coli* indicate possible fecal contamination and threat of health risk
Indicator Hierarchy

Total Coliforms
Widespread: Humans, Animals, Soil

Fecal Coliforms
Animal Sources

E. coli
Thermotolerant (44°C) - Current Standard
IDEXX Technology - Colilert® Systems
Defined Substrate Technology®
Membrane Filtration

- EPA Method 1604 – Total Coliforms and *E. coli* (MI)
- m-ColiBlue – Total Coliforms and *E. coli*
- EPA Method 1603 - Modified Membrane Thermotolerant *E. coli* (mTEC) – *E. coli* only
  - Isolation method used for current TSSWCB-Funded BST projects.
## Considerations

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<th>Colilert ® System</th>
<th>Membrane Filtration</th>
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<td>Minimal training and set-up cost.</td>
<td>More advanced training.</td>
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<td>Pre-Packaged.</td>
<td>Prepared medias available, but more expensive.</td>
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<td>Adequate dilutions yield easy to interpret results.</td>
<td>Overgrowth of non-target organisms can inhibit pure-culture isolations.</td>
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<td>Isolating cultures or confirmatory test requires secondary plating.</td>
<td>Isolations and/or confirmation steps can begin in 24 hours.</td>
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Fig. 1. *E. coli* density determined with specified media as a function of *E. coli* density determined with m-TEC Media. (a) m-ColiBlue24® vs. m-TEC; (b) m-FC-nutrient agar with MUG vs. m-TEC; (c) Colilert® vs. m-TEC.

Hamilton et al. (2005)
Francy and Darner (2000)

Fig. 1. *Escherichia coli* concentrations determined by alternative methods (modified mTEC, MI, and Colilert) compared to the USEPA-recommended method (mTEC) for recreational waters. (The solid line indicates equal recoveries between methods.)
Culture-Independent Analysis of Colilert

*E. coli* Positive Wells

- Non-target taxa made up a substantial percentage of community, especially in creek water (L).

- *Klebsiella* sp. enriched in sewage sample (R).

- 14-23% false positive rates for *E. coli* in creek water.

Sercu et al. (2011)
Comparison of the Diversity of *E. coli* Isolates Obtained from Surface Water Samples using Different Enumeration Methods

- Three standard water quality assessments:
  - EPA Method 1603 - mTEC
  - m-ColiBlue24®
  - Colilert®

- Three sampling locations:
  - Burton Creek, College Station, TX
  - Carter’s Creek Wastewater Treatment Plant (Secondary Treatment), College Station, TX
  - Bryan Lake, Recreational Water Body, Bryan, TX
Materials and Methods

- **Enumeration and Isolation Techniques**
  - ~200 isolates per sampling site per media type.
  - Confirmed via fluorescence on Na-MUG agar.

- **E. coli Community Analysis via ERIC-PCR**
  - Enterobacterial Repetitive Intergenic Consensus sequence-Polymerase Chain Reaction.
  - Products visualized on agarose gel.

- Fingerprint patterns were analyzed with BioNumerics® software.
  - 85% Minimum Similarity Cutoff.
  - Diversity Indices.
**E. coli Diversity Results**

- ERIC fingerprint patterns obtained from both the mTEC (A) and m-ColiBlue24® were more diverse whereas the Colilert® (B) patterns displayed much more clonality.

- Colilert® appears to select for the least diverse *E. coli* populations.
Similarity analysis revealed limited overlap of the isolated communities.

Analysis suggests need for standardization among enumeration and isolation techniques, especially if microbial source tracking applications are anticipated.
Conclusions

- Enzyme-based methodologies yield comparable enumeration results, but not necessarily the same communities.

- Two methods – QuantiTray® vs Membrane offer different ‘habitats’ for growth and thus different communities are selected.

- Choose the enumeration method most fitting to the question being asked.

- Future Research – Incorporate similar study to expand geographic distribution of samples as well as EPA Method 1604 (MI media for detection of both TC and *E. coli*).
Questions?

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