HRSD’s Pathogen Program

• **Core Focus Area**
  - Microbial Source Tracking
  - Quantitative Microbial Risk Assessment
  - Pathogen Quantification

• **Matrices**
  - Stormwater, Biosolids, Wastewater, Drinking Water, Shellfish

• **Capabilities:**
  - Digital PCR
  - Quantitative PCR
  - Next generation sequencing
    - Illumina iSeq 100
    - Nanopore MinION
  - Culture:
    - Traditional FIB
    - Coliphage
    - GB-124
Laboratory Methods

Somatic Coliphage
- EPA Method 1602/1642
- Serial Dilutions

Enteric Viruses
- Electronegative filters
- Adenovirus 41
- Norovirus GI and GII
- Droplet digital PCR
- ATCC Genomic Standards

Somatic Coliphage

25%~75%  Range within 1.5IQR  Median Line  Mean

Coliphage (PFU/mL)

<table>
<thead>
<tr>
<th>dry</th>
<th>wet</th>
<th>dry</th>
<th>wet</th>
<th>dry</th>
<th>wet</th>
<th>dry</th>
<th>wet</th>
<th>dry</th>
</tr>
</thead>
<tbody>
<tr>
<td>upper</td>
<td>lower</td>
<td>pumpstation</td>
<td>raw</td>
<td>effluent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Combined Norovirus GI and GII

25%~75%  Range within 1.5IQR  Median Line  Mean
Human-Associated Fecal Quantitative Polymerase Chain Reaction Measurements and Simulated Risk of Gastrointestinal Illness in Recreational Waters Contaminated with Raw Sewage

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Supporting Information

**ABSTRACT:** We used quantitative microbial risk assessment to simulate the risk of gastrointestinal (GI) illness associated with swimming in waters containing different concentrations of human-associated fecal markers from raw sewage, HF183 and HumM2. The volume/volume ratio of raw sewage to ambient water was determined by comparing marker concentrations in recreational water to concentrations in raw sewage from 54 geographic locations across the United States. Concentrations of reference GI pathogens in raw sewage, volumes ingested by swimmers, dose–response functions, and fractions of infected that become ill were adopted from previous studies. Simulated GI risk increased with concentration of the human quantitative polymerase chain reaction markers in recreational waters. A benchmark illness rate of 30 GI illnesses per 1000 swimmers occurred at median concentrations of 4200 copies of HF183 and 2800 copies of HumM2 per 100 mL of recreational water. This study establishes a risk-based approach for interpreting concentrations of human fecal markers in ambient waters.
Calculating Criteria

![Graph showing the relationship between log median risk and somatic coliphage (log PFU/100 mL). The graph includes a polynomial fit and 95% confidence and prediction bands.](image-url)
Calculating Criteria

![Graph showing the relationship between log median risk and somatic coliphage (log PFU/100 mL). The equation y = Intercept + B1*x^2 + B2*x^2 is given along with the coefficients and R-Square value. The acceptable illness rate is indicated as 30/1000.]
Calculating Criteria

![Graph showing the relationship between log median risk and somatic coliphage (log PFU/100 mL). The equation for the line is $y = \text{Intercept} + B1 \cdot x + B2 \cdot x^2$. The criteria for an acceptable illness rate is set at 30/1000. The table includes the equation details with coefficients and their standard errors.](image)

- **Equation**: $y = \text{Intercept} + B1 \cdot x + B2 \cdot x^2$
  - Intercept: $-2.3143 \pm 0.08254$
  - $B1$: $0.93569 \pm 0.04298$
  - $B2$: $-0.00859 \pm 0.013$
  - R-Square: 0.99323
EPA is Evaluating Coliphage RWQC

- Recommendations/guidance for states to protect users of recreational water
- No new epidemiological studies
- QMRA-based criteria
- The source of sewage affects the calculated criteria
Sources of Sewage to Surface Waters
### Criteria Development Using Location-Specific Data

<table>
<thead>
<tr>
<th>Sewage Source</th>
<th>Data Used</th>
<th>Somatic Criteria (per 100 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw Sewage</td>
<td>wastewater</td>
<td>9</td>
</tr>
<tr>
<td>Final Effluent</td>
<td>disinfected effluent</td>
<td>0.8</td>
</tr>
<tr>
<td>Unknown Infrastructure Issues</td>
<td>upper and lower sewer (wet &amp; dry)</td>
<td>92</td>
</tr>
<tr>
<td>Sanitary Sewer Overflows</td>
<td>wet upper &amp; lower sewer</td>
<td>121</td>
</tr>
<tr>
<td>Lateral Issues</td>
<td>upper sewer (wet &amp; dry)</td>
<td>50</td>
</tr>
<tr>
<td>Force Main Spills</td>
<td>pumpstation (wet &amp; dry)</td>
<td>117</td>
</tr>
</tbody>
</table>
Conclusions

- Different sewage sources have different profiles

- Risk-based criteria development should use sewage source likely to affect local surface waters

- Criteria can vary by several orders of magnitude depending on the data fed in the risk model
Questions?

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