Evaluation of Coliphage as a Beach Water Quality Indicator

John F. Griffith

Presentation to the SCCWRP Commission

September 3, 2021
COLIPHAGE

- Viruses that infect *E. coli*
  - Two main types – Somatic and Male-specific

- Consistently present in high numbers in human sewage

- Easy to enumerate

- Non-pathogenic to humans
WHY MEASURE COLIPHAGE?

- Viruses are often the cause of swimming-associated illnesses in human-impacted waters

- Need an indicator that mimics human viruses
  - Viruses can survive treatment
  - Different fate and transport in the environment

- Coliphage is already approved for other applications
  - EPA Groundwater Rule
  - FDA Shellfish bed monitoring
COLIPHAGE - CURRENT STATUS

• April, 2015 - EPA published Review of Coliphages as Possible Viral Indicators for Fecal Contamination for Ambient Water Quality

• April, 2016 - Coliphages a focus at EPA Recreational Water Conference

• April, 2018 - EPA published Method for Enumeration of Coliphage in Recreational Waters
  – Method 1642: Male-Specific (F+) and Somatic Coliphage in Recreational Waters and Wastewater by Ultrafiltration (UF and Single Agar Layer (SAL) Procedure

• Currently working to establish risk-based thresholds for recreational waters
STATEMENT OF THE PROBLEM

• Need to know if local laboratories can reliably perform the method
  – What level of expertise is required?
  – How much additional time is required for sample processing if method adopted?

• Method has not been tested at California beaches
  – How do coliphage measurements compare to *Enterococcus*?
  – Will we get more frequent hits?
GOALS OF THE STUDY

• Determine “ease of use” of the new coliphage method

• Determine relationship between coliphage and *Enterococcus* at our beaches
APPROACH

• Phase 1: Train participating labs in Method 1642

• Phase 2: Intercalibration exercise to ensure consistency between labs

• Phase 3: Beach Water Testing
  – Initiated August 2019
PARTICIPATING LABS

- Orange County Sanitation District
- Orange County Public Health Lab
- City of Los Angeles
- Los Angeles County Sanitation District
- City of San Diego
- County of Ventura (Cal State Channel Islands)
COLIPHAGE- METHOD 1642

- Ultrafiltration
  - 2L per sample for both MSC and Somatic
  - Hollow-fiber ultrafilter

- Elution from ultrafilter

Single Agar Layer Assay

1. **E. coli host culture**
2. **soft agar w/antibiotics**
3. **Elution solution**
4. **Mix gently, Pour onto plates**
5. **Incubate for 16-24 hrs**
6. **Count plaques**
EVALUATING LAB PERFORMANCE

- **EPA Method Validation Criteria**
  - Percent coliphage recovered from spiked samples
  - Relative percent difference between duplicates

- **Repeatability**
  - Across labs
  - Within labs
MEASURED COLIPHAGE LEVELS FROM SPIKED WATER SAMPLES

+/- 0.5 log group mean
BEACH WATER TESTING

• **12 sites**
  - Samples collected during routine monitoring

• **Wet and Dry weather**
  - 30 samples each site and season

• **Side-by-side measurements of coliphage and *Enterococcus***
<table>
<thead>
<tr>
<th>Monitoring Agency</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>City of San Diego</td>
<td>Imperial Beach</td>
</tr>
<tr>
<td>City of San Diego</td>
<td>Dog Beach</td>
</tr>
<tr>
<td>OCSD</td>
<td>Huntington Bch Stn. 0</td>
</tr>
<tr>
<td>OCSD</td>
<td>Huntington Bch Stn. 3</td>
</tr>
<tr>
<td>LACSD</td>
<td>Inner Cabrillo</td>
</tr>
<tr>
<td>LACSD</td>
<td>Marina Del Rey</td>
</tr>
<tr>
<td>City of Los Angeles</td>
<td>Surfrider Beach</td>
</tr>
<tr>
<td>City of Los Angeles</td>
<td>Santa Monica Pier</td>
</tr>
<tr>
<td>OCPHL</td>
<td>Salt Creek</td>
</tr>
<tr>
<td>OCPHL</td>
<td>San Clement Pier</td>
</tr>
<tr>
<td>County of Ventura</td>
<td>Surfer’s Point at Seaside</td>
</tr>
<tr>
<td>County of Ventura</td>
<td>Surfer’s Knoll Beach</td>
</tr>
</tbody>
</table>
COLIPHAGE LEVELS VARIED BY SITE AND SEASON
PRELIMINARY RESULTS

Dry Season

- Enterococci
- Male-Specific Coliphage
- Somatic Coliphage

Sites in order of median Enterococci concentration
COLIPHAGE LEVELS VARIED BY SITE AND SEASON
PRELIMINARY RESULTS

Wet Season

Enterococci

Male-Specific Coliphage

Somatic Coliphage

Sites in order of median Enterococci concentration
ENTEROCOCCI AND COLIPHAGE LEVELS WERE CORRELATED
PRELIMINARY RESULTS

Dry Season

![Graphs showing correlation between enterococci and coliphage levels during the dry season.](image)
ENTEROCOCCI AND COLIPHAGE LEVELS WERE CORRELATED
PRELIMINARY RESULTS

Wet Season

\[ R^2 = 0.45 \]

\[ R^2 = 0.35 \]
WERE COLIPHAGE LEVELS MEASURED PROBLEMATIC?

- Currently, no EPA threshold

- A recently published risk model suggested thresholds for both somatic and male-specific coliphage in surface waters
  - Somatic: 60 PFU/100 mL
  - Male-specific: 30 PFU/100 mL
SOMATIC COLIPHAGE
PRELIMINARY RESULTS

### Dry Conditions
- Ent [log10(CFU per 100 mL)]
- Coliphage [log10(PFU per L)]
- 8% Ent
- 5% Coliphage

### Wet Conditions
- 21% Ent
- 18% Coliphage

### Summary
- 84% Data Points:
  - Dry: 8% Ent, 5% Coliphage
  - Wet: 21% Ent, 18% Coliphage
- 4% Data Points:
  - Dry: 8% Ent, 5% Coliphage
  - Wet: 4% Ent, 6% Coliphage
- 62% Data Points:
  - Dry: 8% Ent, 5% Coliphage
  - Wet: 62% Ent, 0% Coliphage
- 0% Data Points:
  - Dry: 8% Ent, 5% Coliphage
  - Wet: 0% Ent, 0% Coliphage
MALE-SPECIFIC COLIPHAGE
PRELIMINARY RESULTS

Dry

Wet

Coliphage [log10(PFU per L)]

Ent [log10(CFU per 100 mL)]

10%  30%

3%  8%

87%  0.4%

0.4%  62%

0%  0%
WHAT’S NEXT

• Dry weather sampling will conclude September 30

• Bight Microbiology Committee meeting this month to make a decision on whether to proceed with wet weather sampling

• EPA will soon be making decisions about how and where to implement coliphage
  – SCCWRP will be providing our data to help guide this process