HOW TO MONITOR FOR BACTERIA

James Beckley
Elizabeth Herron
Kris Stepenuck

NWMC Conference
April 28, 2014
WHY MONITOR FOR FECAL BACTERIA

- Fecal bacteria is one of the largest impairment sources of streams
  - For example in Virginia; >9,154 miles
- Waterbodies with fecal bacteria often have other issues such as increased sediment and nutrients
- High levels of fecal bacteria in waterbodies increase the risk of illness

Now on YouTube! Search ‘Attack of the E. coli’ or go to www.youtube.com/watch?v=fyY6YF9xtzc
THE GREAT DEBATE- CFU vs. MPN

- Culture tests report results in CFU or MPN
- CFU or Colony Forming Units is the number of bacteria colonies that formed from the original bacteria in the sample
- MPN or Most Probable Number is a statistical estimate of the number of bacteria that were present in the sample
- There are some minor differences in the two methods but results are essentially the same
- Some states only accept data reported as CFU or MPN
  - A few states get around this by reporting as bacteria per 100 ml
**Escherichia Coli (E. coli)**

- Gram negative, rod shaped, facultative anaerobic bacteria
- Normally found in the digestive tracks of warm blooded animals
- Usually monitored in freshwater
  - Single samples with >235 *E. coli* or a geometric mean of five samples >126 per 100 ml sample indicates excessive bacteria levels
**Fecal Coliform**

- Typically found in the digestive tract of warm blooded animals
  - *E. coli* and other species are members
- Most states have moved to sampling for *E. coli* or Enterococcus
- Fecal coliform monitoring still required in shellfish waters
  - Average levels must be <14 and single maximum readings no more than 49 MPN or 31 CFU
ENTEROCOCCUS

- Gram positive, spherical shaped, facultative anaerobic bacteria
- Normally found in the digestive tracks of warm blooded animals
- Usually monitored for in saltwater (beaches)
- Excessive bacteria levels typically indicated by:
  - Salt water samples with >104 enterococci or an average >35 per 100 ml sample
  - Freshwater samples with >61 enterococci or an average >33 per 100 ml sample
POPULAR METHODS TO MONITOR FOR FECAL BACTERIA

- Presence/Absence
- Coliscan Easygel
- 3M Petrifilm
- LaMotte BioPaddle
- Colilert/Enterolert
- Membrane Filtration
- Multiple Tube Method
PRESENCE/ABSENCE

- Simplest culturing method
- Sample placed in a bottle containing nutrient media
- Color change/glow under UV or similar reaction indicates presence of the bacteria
- Results in 24 hours
- Normally used to test drinking water quality as no fecal bacteria should be present
- Can be used for education/awareness
COLISCAN EASYGEL

- Does not require a laboratory
- Tests 1 to 5 ml of sample
- Results in 24 to 48 hours
- *E. coli* appear dark blue to purple
- Results are in CFU
- Good to locate bacteria ‘hot spots’ and track overall trends

1 ml sample with 5 *E. coli* colonies
3M PETRIFILM

- Non-laboratory based test to detect *E. coli* and other coliform bacteria
- Tests 1 ml of sample but can be coupled with membrane filtration to test 100 ml
- *E. coli* appear dark blue with gas bubbles
- Results are in CFU
- Good for screening and education/awareness
LaMotte Bio Paddles

- Non-laboratory based test to detect
  - Total coliform
  - Fecal coliform
  - E. coli
- Can be used to Calculate Total Viable Count and Total Colony Count from a 40 ml sample volume (for counts <300)
- Estimate colony density >300
- CFU results
- Good for screening and education/awareness
C olilert/Ent erolert

- Most popular lab-based method due to relative low cost and ease of use
- Results in 18 to 24 hours

- Colilert
  - Total coliform wells turn yellow
  - *E. coli* wells are yellow AND glow under a UV

- Enterolert - Enterococcus positive wells glow under a UV light but no color change

- Results in MPN
- Test is susceptible to optical brightener/pesticide interference
Membrane Filtration

- Filter sample through a fine pore (< 0.6 um) filter
- Tests up to 100 ml of sample
- Results in 18 to 24 hours
- Colony color depends on nutrient media
- Results reported as CFU
- Requires autoclave and vacuum pump and related laboratory equipment
**Multiple Tube Method**

- Not used as much since introduction of Colilert/Enterolert
- Multiple test tubes of media/auger inoculated with several sample volumes
- Results in 3 to 5 days
- Number of test tubes with a positive reaction determines the MPN result
<table>
<thead>
<tr>
<th>Test Method</th>
<th>Presence/Absence</th>
<th>Coliscan Easygel</th>
<th>3M Petrifilm</th>
<th>LaMotte Bio-Paddle</th>
<th>Colilert/Enterolert</th>
<th>Membrane Filtration</th>
<th>Multiple Tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab Required</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Min Detection Limit</td>
<td>0</td>
<td>&lt;20</td>
<td>&lt;100</td>
<td>&lt;40</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Max Detection Limit</td>
<td>1</td>
<td>&gt;6000</td>
<td>&gt;6000</td>
<td>&gt;1,000,000</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>Unit of Measurement</td>
<td>N/A</td>
<td>CFU</td>
<td>CFU</td>
<td>CFU</td>
<td>MPN</td>
<td>CFU</td>
<td>MPN</td>
</tr>
<tr>
<td>Consumable Cost</td>
<td>$6.50</td>
<td>$2.50</td>
<td>$3.00</td>
<td>$6.00</td>
<td>$6.50</td>
<td>$2.50</td>
<td>$2.00</td>
</tr>
<tr>
<td>Equipment Cost</td>
<td>$0</td>
<td>$100</td>
<td>$100</td>
<td>$100</td>
<td>$15,000</td>
<td>$25,000</td>
<td>$25,000</td>
</tr>
<tr>
<td>Time Spent Per Sample</td>
<td>1 min</td>
<td>1 min</td>
<td>1 min</td>
<td>&lt; 1 min</td>
<td>5 min</td>
<td>&gt;10 min</td>
<td>&gt;10 min</td>
</tr>
<tr>
<td>Incubation Time (Hrs)</td>
<td>24</td>
<td>24 to 48</td>
<td>24</td>
<td>18 to 24</td>
<td>18 to 24</td>
<td>18 to 24</td>
<td>72 to 120</td>
</tr>
<tr>
<td>EPA Approved</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Effective Monitoring Strategies

- “Shotgun” method
- Targeted source identification/verification
- Carpet sampling (“B-52” method)
- Routine sampling to assess baseline conditions
- Storm event sampling
SHOTGUN METHOD

- Sample stations randomly scattered in the watershed
  - Sampling from public access points like bridges
- Ideal for initial recon to find bacteria “hotspot” segments
- Moderate labor and cost intensive
TARGETED SAMPLING

- Sample likely sources of fecal bacteria
  - Sewer line crossings or near septic systems
  - Areas of known dense wildlife or animal activity
- Good to quickly rule out or confirm fecal sources
- Requires planning to identify sources and may require landowner access

<table>
<thead>
<tr>
<th>Date</th>
<th>Type</th>
<th>E.Coli /100ml water</th>
</tr>
</thead>
<tbody>
<tr>
<td>3/15</td>
<td>dry</td>
<td>14,900</td>
</tr>
<tr>
<td>3/29</td>
<td>600</td>
<td>20,900</td>
</tr>
<tr>
<td>4/25</td>
<td>dry</td>
<td>&gt;20,000</td>
</tr>
<tr>
<td>5/28</td>
<td>3,800</td>
<td>&gt;30,000</td>
</tr>
<tr>
<td>6/19</td>
<td>Sewer repaired</td>
<td></td>
</tr>
<tr>
<td>6/26</td>
<td>dry</td>
<td>200</td>
</tr>
<tr>
<td>8/16</td>
<td>100</td>
<td>300</td>
</tr>
</tbody>
</table>
CARPET SAMPLING

- Sampling every possible source and waterbody segment at one time
- Extremely labor and cost intensive
- Provides the most detailed picture of fecal sources in the shortest amount of time
- No E. coli violations

- Several E. coli violations (storm events)

- Constantly high (<50%) E. coli Counts
Routine Monitoring

- Sampling part of overall monitoring strategy
- Can be used to assess baseline conditions, or target recreational waters not otherwise monitored
- Minimal additional effort required, but sample holding times can become a concern
STORM EVENT SAMPLING

- Sampling at the first stages of a major rainstorm
  - Shows the worst case scenario of site bacteria levels
  - Helpful in identifying sources of bacteria not readily seen during dry sample events
- Most effective if samples are collected within the first hour of a storm
- >¼” rainfall is often enough to elevate bacteria levels
- Storm event sampling can be a part of any plan
**Before Heading Out**

- Let someone know you are heading out and when you expect to be back
  - If possible, have a co-sampler join you
- Check the weather
  - Dress appropriately
- Inspect equipment
  - Check condition of coolers, sample bottles, marker, sample bucket, etc.
  - Have backup sample bottles
  - Have safety equipment (first aid kit, flares, etc.)
IF SAMPLING ALONG A ROAD

- If a safe parking area away from the road is not available, pull completely off the road and onto the shoulder
  - Park away from sharp curves
  - Leave sufficient room to exit/enter the vehicle
  - Turn on the vehicle hazard signal
  - If possible, wear a reflective or orange safety vest
  - Try to exit away from the path of motor vehicles
**Bridge Safety**

- Be aware of traffic conditions
- If there is a no loitering or fishing sign, best not to sample on the bridge
- Sample on the upstream or the safest side of the bridge
**CONTAMINATED WATER**

- Use extra protection if water has an unusual odor or color
- Wear gloves or have hand sanitizer available during sampling
  - Remember you can contaminate the sample too!
- Wash or disinfect hands after sampling and prior to eating
- Always assume sample water contains pathogens!
Exercise Time