OVERVIEW

- State freshwater fecal coliform criteria
- KCEL - Microbial Source Tracking (MST) ‘tool kit’
- Summary of findings for Juanita Phase II and Phase III
Most routinely monitored streams

- Extraordinary Primary Contact Criteria
- exceed part 1 of criteria

Source control is a challenge:

- FC exist in the gut of all warm-blooded animals
- FC proliferate in the environment

<table>
<thead>
<tr>
<th>State Freshwater Criteria</th>
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<tbody>
<tr>
<td>Extraordinary Primary Contact</td>
<td>Primary Contact</td>
</tr>
<tr>
<td>single sample*</td>
<td>single sample*</td>
</tr>
<tr>
<td>&gt;100 CFU/100 ml</td>
<td>&gt;200 CFU/100 ml</td>
</tr>
<tr>
<td>GEOMEAN</td>
<td>&gt; 100 CFU/100 ml</td>
</tr>
<tr>
<td>&gt; 50 CFU/100 ml</td>
<td>&gt; 100 CFU/100 ml</td>
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</tbody>
</table>

* or 10% of samples if more than 10 sample points

Percent summer (June – Sept) samples 1986 – 2012

> 100 CFU/100 ml

number of samples collected: most (108) ➔ least (3)
KCEL Microbial Source Tracking “tool kit”

- Fecal coliform (FC) - state water quality standard
- *Escherichia coli* (E. coli) - part of the group of fecal coliforms (EPA recommended)

- Sorbitol-fermenting *Bifidobacteria* - human gut bacteria
  Reported as presence/absence

- *Rhodococcus coprophilus* - pasture bacteria excreted by grazing animals
  Reported as presence/absence

- Human *Bacteroides* qPCR – human gut bacteria
  Reported as cells/100 mls

- Ruminant *Bacteroidales* PCR – targets sheep and cattle
  Reported as presence/absence
sorbitol-fermenting *Bifidobacteria*
present/absent

**PROS**
- constitute large percentage of human gut flora & in higher numbers than fecal coliforms
- human sorbitol fermenting - easily distinguishable from other sources
- do not proliferate in the environment
  - strict anaerobes
- short survival - makes excellent indicators of recent contamination

**CONS**
- requires media prep, special preservation and collection
- requires 8 days to complete
- negative results should be interpreted with care
**PROS**

- Comprise ~50% by weight of the bacterial biomass in the feces of humans.
- Do not proliferate in the environment
  - Strict anaerobes
  - Optimal growth ~ 37°C
- Potentially quantitative microbial source tracking tool.

**CONS**

- DNA persists for up to one week after cell death
- Uncertainty around lower threshold for human contamination
- Need for relative scale based on known pollution sources
**Rhodococcus coprophilus**

*present/absent*

**PROS**
- found in the manure of domesticated grazing animals
- never been isolated from human feces.

**CONS**
- culture based – requires media prep in advance
- slow grower – 2-3 weeks for results
- pasture bacteria – may not be present when domesticated animals are not grazing
ruminant *bacteroidales* PCR

**PROS**

- Does **not** rely on feeding habits of the animal
- Specific to sheep and cattle
- Positive result indicates fecal contribution from a non-human source

**CONS**

- Does not detect fecal contamination from other farm associated grazers such as pigs, horses, chickens, alpaca, goat, etc.
- Results to date only represent a small sample set in 2012
**MST Lines of Evidence**

Fecal Coliform

<table>
<thead>
<tr>
<th>State Recreational Criteria for Fecal Coliform Bacteria</th>
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</thead>
<tbody>
<tr>
<td><strong>Extraordinary</strong> single sample</td>
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<tr>
<td>&gt;100 CFU/100 ml</td>
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<thead>
<tr>
<th>Ten State Swimming Beach Criteria</th>
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</thead>
<tbody>
<tr>
<td><strong>fecal coliform</strong></td>
</tr>
<tr>
<td>&gt; 1000 CFU/100 ml</td>
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<table>
<thead>
<tr>
<th>Other</th>
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<tbody>
<tr>
<td><strong>EPA Recommended</strong></td>
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<tr>
<td>E. coli</td>
</tr>
<tr>
<td>&gt; 235 CFU/100 ml</td>
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</tbody>
</table>
2011 Pilot MST Projects

All are 303(d) listed for fecal coliform bacteria

- Idylwood Creek – creek flows into swimming beach area
- Juanita Creek – swimming beach closures and potential TMDL
- Boise Creek - TMDL
- Issaquah Creek – TMDL
- White Center - Hicks Lake
Intensive FC Bacteria Survey

- Ecology
- City of Kirkland
- King County

“Direct Implementation”
rather than begin TMDL Process

Action Plan:
1. Identified areas of concern
2. -Ecology monthly sampling in selected areas
3. -Investigate MST options

Main stem of the creek highlighted
Juanita Creek Phase II ~ 2011 MST

- Ecology – monthly sampling
- City of Kirkland
- King County Environmental Lab

- Billy Creek
  All 4 indicators
  Direct discharge to creek

- North Fork
  FC, E.coli, qPCR, some bifidobacteria

- West Fork
  FC, E.coli, qPCR
AM/PM
June - Sept

- No bifidobacteria
- High FC and E. coli, qPCR
Three lines of evidence:

- Dropped E. coli (cost savings)
- Sampled three consecutive days, AM & PM
  - July 30 – Aug 1
  - Aug 20 - 22

City of Kirkland
King County

- No bifidobacteria
- FC relatively low

- City of Kirkland
- King County
- No bifidobacteria
- PM FC signal - July 30
- qPCR signal late Aug
- City of Kirkland
- King County
Juanita Creek ~
Next Steps

1. Kirkland work with Northshore Utility District to investigate history of sewer hook ups in the areas of concern in North Fork segments

2. Follow up MST testing upper Billy Creek

3. Investigate upstream of West Fork using the MST Tool Kit
1. Are FC and qPCR bacteroides an effective MST Tool Kit alone?
   - As a cost savings is it useful to collect and preserve a qPCR filter with each FC for later analysis dependent upon high FC values?

2. What scale of human bacteroides qPCR values triggers further investigation?
   - Limited literature shows qPCR values in the 100,000 range indicates direct sewage source. Do we put resources towards chasing values that are less than six digits?

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