Progress in Microbiology

Presentation to the SCCWRP Commission
September 11, 2015

John F. Griffith
SCCWRP IS A WORLD LEADER IN MOLECULAR TECHNOLOGY FOR BEACH WATER QUALITY

- **Method Development**
  - Identifying and adapting the best new technology for use by member agencies

- **Method Evaluation**
  - Able to bring leading method developers together
  - Serve as an “honest broker”

- **Implementation and Technology Transfer**
  - Train member agencies and others
  - Aim is to get the best methods into the hands of member agencies
THREE MAIN AREAS OF RESEARCH

• Rapid Methods

• Microbial Source Identification

• Epidemiology Studies and Risk Modeling
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RAPID MICROBIAL MEASUREMENT METHODS

• Culture methods are too slow for same day warnings

• SCCWRP has been instrumental in development and adoption of qPCR as a rapid method
  – Served as EPA’s West Coast partner
  – Trained 14 labs (including all of our POTW member agencies)
  – Demonstrated ability to provide information to beachgoers within 4 hours

• Working collaboratively to evaluate qPCR across different beach types as part of the Bight program
  – Conducted lab intercalibration
  – Serve as Help Desk for group
WHY IS FIELD PORTABLE SO IMPORTANT?

• **Beach water quality**
  - Need an answer by noon
  - Not realistic to send individual samplers to every beach

• **Microbial source tracking**
  - Sources of contamination are often ephemeral
  - Need to be able to track contamination back to source
WE HAVE A PROTOTYPE INSTRUMENT

- Field Portable

- Same basic technology as old method

- Could be mounted in a land or automated underwater vehicle or operated by a lifeguard
**DIGITAL PCR**

Old Method

- 20µl PCR reaction
- Unknowns plus standards
- Compare to Standard Curve

Digital PCR

- 20µl PCR reaction
- 10000 - 20000 droplets or chambers
- Direct quantification using statistics
HIGHLY CORRELATED WITH CURRENT METHOD

![Graph showing correlation between new and old methods for Enterococcus count. The correlation coefficient (R^2) is 0.97.](image)
ADDITIONAL ADVANTAGES

• Solves many of the inhibition issues associated with qPCR

• Greatly reduces the potential for false negative results
## RESISTANT TO INHIBITION

<table>
<thead>
<tr>
<th>Humic acid concentration (ng/µl)</th>
<th>Old Method (gene copies)</th>
<th>New Method (gene copies)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1810</td>
<td>1810</td>
</tr>
<tr>
<td>1</td>
<td>1165</td>
<td>1680</td>
</tr>
<tr>
<td>2.5</td>
<td>184</td>
<td>1700</td>
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<tr>
<td>5</td>
<td>0</td>
<td>1870</td>
</tr>
</tbody>
</table>
NEXT STEPS

• Presently testing instrument in the lab

• Will start field trials next Spring
THREE MAIN AREAS OF RESEARCH

• Rapid Methods

• Microbial Source Identification

• Epidemiology Studies and Risk Modeling
MICROBIAL SOURCE IDENTIFICATION

• When you have a problem, you want to know the source

• Need to be able identify sources of bacteria and extent of contamination
  – Can expend a lot of resources without fixing the problem
MICROBIAL SOURCE ID RESEARCH

- SCCWRP a national leader in microbial source identification

- Conducted largest Microbial Source Identification Method Evaluation Study
  - Included all the top researchers in the US and Europe
  - Dedicated issue of *Water Research*
  - Achieved broad scientific consensus regarding best methods

- Microbial Source Identification Manual
  - Bible for source ID work
  - State has adopted for all Bond funded work
REMAINING CHALLENGES

• **Studying relative degradation of microbes**
  – Source associated markers
  – Fecal indicator bacteria
  – Pathogens

• **Need understand how markers behave in the environment**
  – All previous work has been done in fresh water

• **Developing a model to help interpret results**
Dialysis bag containing ambient water and 5% v/v sewage

Shade cloth covering half of experiment
Freshwater (Irvine)

Brackish water (Santa Barbara)

- 10 day deployments
- Summer and winter
- Full sun or shade

Ocean water (Pillar Point Harbor)
<table>
<thead>
<tr>
<th>Category</th>
<th>Analysis</th>
<th>Sewage</th>
<th>Cattle</th>
<th>Gull</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FIB</strong> (by culture or PCR)</td>
<td>Enterococcus (culture)</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>E. coli (culture)</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Enterococcus (PCR)</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>General Bacteroidales (PCR)</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td><strong>MST markers</strong> (by PCR)</td>
<td>HF183 (human)</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HumM2 (human)</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BacHum (human)</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CowM2</td>
<td></td>
<td>X</td>
<td></td>
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<tr>
<td></td>
<td>Catellicoccus (gull)</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td><strong>Pathogens</strong> (by culture and PCR)</td>
<td>Norovirus (RT-PCR)</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Campylobacter (culture)</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Salmonella (culture)</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Campylobacter (PCR)</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Salmonella (PCR)</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td><strong>Community analysis</strong></td>
<td>PhyloChip (microarray)</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Illumina (sequencing)</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
PRELIMINARY RESULTS

Under shade, all molecular targets

Cow

Gull

Sewage

log10 Copy per 100ml

Day

Analyte.O
- E. coli
- Enterococcus
- Bacteroidales, Total
- Bacteroidales, Human
- Bacteroidales, Cow
- Catellicoccus
- Campylobacter
- Salmonella

as.factor(AnalyteCat)
- FIB
- MST
- Pathogen
NEXT STEPS

• Field studies complete

• Conducting data analysis
  – Manuscripts for CTAG in 6 months

• Model available
  – Summer 2016
THREE MAIN AREAS OF RESEARCH

• Rapid Methods

• Microbial Source Identification

• Epidemiology Studies and Risk Modeling
EPIDEMIOLOGY STUDIES

• Need to have thresholds in order to interpret results from new markers
  – What level is important for health risk?

• Epidemiology studies determine thresholds

• SCCWRP has been a national leader in epidemiology studies
BASICS OF EPIDEMIOLOGY

• Recruit swimmers and non-swimmers to participate in study

• Take concurrent water quality measurements
  – Need to know levels of indicators/markers at time of exposure

• Compare illness rates between exposed and unexposed participants
  – Difference (if any) attributed to water exposure

• Determine if there is a relationship between illness and measured levels of indicators/markers
CHALLENGES OF A WET WEATHER STUDY

• Not as many folks swimming in the winter
  – Usually recruit at beach and follow up by phone
  – Must use exposure days to make up for small sample size

• Makes field operations more difficult
  – Rain
  – Big surf
  – Fast moving water
STRATEGY

• Targeted 2 popular surfing beaches in San Diego
  – Tourmaline Surfing Park
  – Ocean Beach

• Followed health throughout study (16 weeks) in wet and dry weather
  – Developed phone and web apps
  – Provided incentives for staying in study

• Daily beach water quality measurements for duration of study
## EXPOSURE DAYS

<table>
<thead>
<tr>
<th>Location</th>
<th>Subjects Enrolled</th>
<th>Total Days of Follow-up</th>
<th>Ocean Exposure Events</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Surfer Health Study</strong></td>
<td>654</td>
<td>33,377</td>
<td>10,081</td>
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<tr>
<td>Boqueron, PR</td>
<td>15,726</td>
<td>172,986</td>
<td>12,111</td>
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<tr>
<td>Surfside, SC</td>
<td>11,159</td>
<td>122,749</td>
<td>8,073</td>
</tr>
<tr>
<td>Silver, MI</td>
<td>10,921</td>
<td>120,131</td>
<td>5,651</td>
</tr>
<tr>
<td><strong>Mission Bay, CA</strong></td>
<td>12,469</td>
<td>137,159</td>
<td>4,524</td>
</tr>
<tr>
<td>Doheny, CA</td>
<td>9,525</td>
<td>104,775</td>
<td>4,335</td>
</tr>
<tr>
<td>Avalon, CA</td>
<td>6,165</td>
<td>67,815</td>
<td>3,891</td>
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<tr>
<td>Malibu, CA</td>
<td>5,674</td>
<td>62,414</td>
<td>2,559</td>
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<tr>
<td>Washington Park, IN</td>
<td>4,377</td>
<td>48,147</td>
<td>2,360</td>
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<tr>
<td>West, IN</td>
<td>2,877</td>
<td>31,647</td>
<td>1,668</td>
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<tr>
<td>Goddard, RI</td>
<td>2,977</td>
<td>32,747</td>
<td>1,080</td>
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<tr>
<td>Fairhope, MS</td>
<td>2,022</td>
<td>22,242</td>
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<tr>
<td>Huntington, OH</td>
<td>2,840</td>
<td>31,240</td>
<td>757</td>
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<tr>
<td>Edgewater, AL</td>
<td>1,351</td>
<td>14,861</td>
<td>741</td>
</tr>
</tbody>
</table>
WET VS. DRY EXPOSURE

Tourmaline
Ocean Beach
All SD Beaches

Wet
Dry
NEXT STEPS

• Field work complete

• Data analysis underway

• Expect a report in early 2016
QUANTITATIVE MICROBIAL RISK ASSESSMENT

- Less expensive alternative to epidemiology study

- EPA has established method
  - 2012 Recreational Water Criteria

- We are conducting a prototype
WET WEATHER QMRA STRATEGY

Measure pathogens in stormwater

Bacteria, Viruses & Protists

Estimate exposure

Infective dose

Risk Assessment

Predict Health Risk and compare with Health outcomes
# WHAT WE ARE MEASURING

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus</td>
<td>Culture and PCR</td>
</tr>
<tr>
<td>Coliphage</td>
<td>Culture</td>
</tr>
<tr>
<td>Campylobacter</td>
<td>PCR</td>
</tr>
<tr>
<td>Salmonella</td>
<td>PCR</td>
</tr>
<tr>
<td>Norovirus</td>
<td>PCR</td>
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<tr>
<td>Adenovirus</td>
<td>PCR</td>
</tr>
<tr>
<td>Enterovirus</td>
<td>PCR</td>
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<tr>
<td>Giardia</td>
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<tr>
<td>Cryptosporidium</td>
<td>PCR</td>
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<tr>
<td>Human Marker</td>
<td>PCR</td>
</tr>
<tr>
<td>Dog Marker</td>
<td>PCR</td>
</tr>
<tr>
<td>Gull Marker</td>
<td>PCR</td>
</tr>
</tbody>
</table>
NEXT STEPS

• Field work complete

• Data analysis underway

• Epidemiology will show if QMRA is working

• Embarking on a dry weather QMRA study at Cabrillo Beach
SCCWRP is a world leader in molecular technology for beach water quality

- **Method Development**
  - Identifying and adapting the best new technology for use by our member agencies

- **Method Evaluation**
  - Able to bring leading method developers together
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