Parasitic Surveillance Practices for Water Utilities

Cryptosporidium and Giardia

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Outline

- NYC water supply
- Historical protozoan methods
- Routine sampling method
- Risk
- Method options
- Supplemental tests
- Summary
New York City Water Supply

- Primarily a surface water supply
- 19 reservoirs & 3 controlled lakes
- System Capacity: 570 billion gallons (2,158 billion liters)
- Serves 9.6 million people (1/2 of population of New York State)
- Delivers approx. 1.1 billion gallons (4.2 billion liters) per day
- Source of water is a 2,000 square mile (5,180 square kilometer) watershed in parts of 8 upstate counties
- Operated and maintained by the New York City Department of Environmental Protection (NYCDEP)
Protozoan Monitoring Locations

Source Water

Pre-finished Water

OFF-LINE

= sampling locations
### Protozoan Method History

26 years; 3 methods; 3 laboratories

<table>
<thead>
<tr>
<th>Years</th>
<th>Sites</th>
<th>Methods</th>
<th>Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>1992 - 1999</td>
<td>Keypoints</td>
<td>ASTM</td>
<td>NYS DOH/ ECWA</td>
</tr>
<tr>
<td>1999 – Oct 2001</td>
<td>Keypoints</td>
<td>ICR</td>
<td>NYC DEP</td>
</tr>
<tr>
<td>Oct 2001 – Apr 2015</td>
<td>Keypoints</td>
<td>1623HV</td>
<td>NYC DEP</td>
</tr>
<tr>
<td>Apr 2015 – current</td>
<td>All sites</td>
<td>1623.1 + EasyStain</td>
<td>NYC DEP</td>
</tr>
<tr>
<td>Mar 2016</td>
<td>Hillview</td>
<td>1623.1 + EasyStain Heat</td>
<td>NYC DEP</td>
</tr>
<tr>
<td>Aug 2017</td>
<td>All sites</td>
<td>1623.1 + EasyStain Heat</td>
<td>NYC DEP</td>
</tr>
</tbody>
</table>

ECWA = Erie County Water Authority
US EPA Method 1623/ 1623.1

Elution

IMS separation

Concentration

Slide staining
Routine - Method 1623/ 1623.1 Microscopy (50L)

- **DOES:**
  - Detect *Cryptosporidium* spp. and *Giardia* spp.
  - Identify internal structures, if present (DAPI nuclear stain, DIC)

- **DOES NOT:**
  - Determine genotype, viability, or infectivity
    - Not all oocysts, even if viable and infectious, have the ability to cause illness in humans
    - Most oocysts found in the environment are not species or genotypes that cause illness in humans
Public Health Risk?

- Are there any detected?
  - Few or many?
- Where are they?
  - the upper watershed?
  - the source water?
  - the treated water?
- Are they types known to cause human illness?
- Are they viable/infectious?
1623/ 1623.1 Method Options

- **Stain**
  - Merifluor: 2001-2015
  - EasyStain: 2015 to present – improved *Cryptosporidium*

- **Dissociation**
  - Acid: 2001-2015
  - Heat: 2016 – improved *Giardia*

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*G. duodenalis* using EasyStain

*G. muris* using EasyStain
Hillview Site 3: *Cryptosporidium* MS

Method 1623 + Merifluor

Method 1623.1 + ES

Method Acid to Heat

% Cryptosporidium Recovery

2013 (n = 15)  
2014 (n = 39)  
2015 (n = 56)  
2016 (n = 50)  
2017 (n = 33)
Hillview Routine Sampling Summary

- Weekly sampling - August 2011 (Method 1623/1623.1, 50L)
  - 21/394 (5.3%) samples positive (two = 2 oocysts)
  - 23/19,700 liters = 0.00117 oocyst/L

<table>
<thead>
<tr>
<th>Year</th>
<th># Detects</th>
</tr>
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<tbody>
<tr>
<td>2011</td>
<td>0</td>
</tr>
<tr>
<td>Aug-Dec</td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>0</td>
</tr>
<tr>
<td>2013</td>
<td>2</td>
</tr>
<tr>
<td>2014</td>
<td>2</td>
</tr>
<tr>
<td>2015</td>
<td>6</td>
</tr>
<tr>
<td>2016</td>
<td>4</td>
</tr>
<tr>
<td>2017</td>
<td>2</td>
</tr>
<tr>
<td>2018</td>
<td>5</td>
</tr>
</tbody>
</table>

Graph showing Cryptosporidium oocysts detections from 2011 to 2019.
Cryptosporidium – Source v. Treated

<table>
<thead>
<tr>
<th>Site</th>
<th>Location</th>
<th>Crypto # pos./ n</th>
<th>Percent detect</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEL18DT</td>
<td>Source</td>
<td>5/ 55</td>
<td>9.1%</td>
</tr>
<tr>
<td>CCCLAB</td>
<td>Post-UV</td>
<td>7/ 54</td>
<td>13.0%</td>
</tr>
<tr>
<td>HV Site 3</td>
<td>Pre-treated</td>
<td>5/ 55</td>
<td>9.1%</td>
</tr>
</tbody>
</table>

Cryptosporidium at Site 3 is likely UV inactivated and of no risk
Cryptosporidium Genotyping

- Positive Hillview slides are submitted for typing
  - Recovery from slide – WRF 4099 (2010)
    - CDC&P - SSU rRNA-based PCR RFLP
    - University of Texas Public Health Lab – multiplex PCR, hsp70
    - NYC DEP Pathogen Laboratory
  - DNA sequencing as needed
  - All 21 positive samples at Hillview were negative for Cryptosporidium
  - UV treatment alters DNA interfering with PCR, further suggesting these oocysts have been UV inactivated
- Cell-culture Immunofluorescent Assay (CC-IFA) – SM 9711D
- Method 1623 up to IMS bead dissociation
- Dissociate beads using Acidified (pH 2.0) Hanks Balanced Salt Solution with Trypsin (AHBSS/T)
- Add cell culture medium and remove AHBSS/T
  - Centrifuge and aspirate
- Inoculate HCT-8 cell monolayer
- Centrifuge well plate and incubate
- Direct stain – Sporo-Glo
All CC-IFA samples collected weekly at the outflow of Hillview Reservoir have been negative for infectious *Cryptosporidium* (since May 2018)
Summary

- NYC DEP monitors numerous streams, reservoirs and aqueducts throughout the 2000sq mi watershed.
- NYC DEP has studied and selected method options to improve recovery (1623.1+ EasyStain, heat dissociation).
- US EPA method (1623.1) is effective for the recovery of *Giardia* and *Cryptosporidium*; however overestimates public health risk (post-UV positives).
- *Cryptosporidium* genotyping and CC-IFA infectivity testing have been added to better inform public health risk.
- Similar source water and post-UV concentrations, PCR negative slides and no infectious oocysts indicate low public health risk.
- Molecular techniques identifying (oo)cysts beyond genus and species are critical for the refined assessment of protozoan risk to public health.
For more information...

Visit the DEP website at www.nyc.gov/dep

Follow us on Facebook for more info about events and projects, photos and other watershed updates: facebook.com/nycwatershed