New Contaminants Are Always Emerging

High-Throughput Methods for Testing Serum and Drinking Water

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Wadsworth Center
NY State Department of Health
Why are “New Contaminants Always Emerging?”

- Sensitive Analytical Instrumentation
- Rapid Data Acquisition and Processing
- Untargeted Analysis

- New Compounds being introduced into Commerce
- Accepted releases into the Environment
- Uncontrolled releases into the Environment

- Personal Car Products - Exposure Routes
NY State Public Health Lab

Response to Communities with Emerging Contaminants in Drinking Water.

PFOA, PFOS
Driving Forces – Drinking Water

- Known levels of PFAS in Public Drinking Water Supplies for some NYS communities
- May 19th, 2016 – EPA issues lifetime health advisory of 70 ppt for long-term exposure
- Monitoring PFOA and PFOS – NY State Assembly Budget Proposal (FY 2017-2018)
  - Requires NYS Health Commissioner to identify and monitor emerging contaminants in PWS specifically including PFAS and 1,4-Dioxane.
Driving Forces – Public Health Monitoring

• Intense public interest in body burden assessment
• PFAS included in NHANES biomonitoring (comparative purposes)
• Provide public health service, with accurate and defensible data (CLIA compliant), to NYS residents
• Biomonitoring database for evaluation of remedial actions.
• Improve outreach/partnerships with public hospitals and Public Service Centers within NYS
• Assist neighboring states through testing (NH and VT) or disseminating knowledge (MA)
Components of a High-Throughput Method

Key components of each (bio)monitoring project involve sections of the PH organization that has to work together to allow efficient sample flow consistent with maintaining data quality.

- Trained staff with sampling equipment pre-checked for contamination available at collection site.
- Data entry for sample analysis request forms
- Sample collection and transport to laboratory
- Rapid robust instrumental analytical procedures
- Approved Standard Operating Procedures (SOPs)
- Efficient Analytical Data review and reporting
- Secure Data reporting (clinical sample reports)
Laboratory Response Network (LRN-C) and Public Health Response

What constitutes a LRN-C Laboratory?

• LRN-C infrastructure, assets + prepared.
  • Skilled analysts – method development
  • Analytical instrumentation
  • Biological sample logistics
  • Sample collection and shipping
  • Automated sample processing
  • CLIA Compliant
  • Surge capacity training
  • Rapid results reporting
  • Outreach
Ongoing LRN-C Developments and Goals

Expand Laboratory capability and capacity for Public Health Response.

- Human Biomonitoring - Exposure Assessment
- Method Development
- High throughput sample preparation
- Advanced analytical instrumentation
- Improved data tracking and reporting
- Identify opportunities for increased efficiencies
- Handling emerging potential exposure concerns
Recent NY Lab activities
Recent Chemical Public Health Issues in NY

• Three initiated projects:-
  1. Drinking water contamination in NE States with Perfluoroalkyl Substances (PFAS) Exposure Assessment (Biomonitoring)
  2. Emerging Water Contaminants (EPA UCMR4)
     1,4-Dioxane (EPA Method 522) + “Untargeted testing”
  3. Medical Marijuana (State program support)
     Synthetic Drugs
     Hemp?
PFAS Testing in Drinking Water

- ISO Method 25101:2009(E) was validated for the testing of Drinking Water using automated solid phase extraction and LC/MS/MS. Over 500 water samples have been analyzed from Hoosick Falls during the initial part of this public health project.
- NY Environmental Laboratory Accreditation Program (ELAP) is providing interim approval for commercial labs to test drinking water using this ISO method.
## Water PFAS Analysis

### Water sample pretreatment

**SPE Extraction using Thermo Dionex Autotrace 280**

- Weigh collected samples
- Dispense 200 µL methanol internal 100ppb standard solution into each collected sample
- Load about 220 to 250 mL of sample onto Waters brand Oasis WAX 6 cc Cartridge, 150 mg Sorbent using Thermo Dionex Autotrace 280
- Weigh empty bottles and subtract from full weight. Elute with 0.1% Ammonium Hydroxide/Methanol
- Collect elutates from Autotrace, and concentrate samples down to 1mL using nitrogen.
- Transfer 1mL samples to 1.5ml vials and put on UPLC/MS/MS system for analysis

### Quantitative Analysis

**Liquid Chromatography – MS/MS with isotope dilution quantitation.**

- Agilent 1290 UHPLC system, Agilent 6460 triple quadrupole mass spectrometer equipped with electrospray ionization source.
- Run time: 6 min
- Flow rate: 0.3 mL/min
- Mobile Phase: MeOH/ 0.1% Ammonium Acetate
- Waters Acquity UPLC BEH C18 1.7 µm 2.1 x 50mm column or equivalent
- MRM scan with Negative polarity
Automated SPE and Agilent LC/MS/MS for PFAS analysis of Water Samples

Thermo Autotrace 280
Public Water Supply – PFOA contamination

• 2016 - Hoosick Falls, NY

• The Village of Hoosick Falls is located in the Town of Hoosick, a rural community located in northeastern Rensselaer County, about 30 miles from Albany. The Town has a population of 6,700 with 3600 living in the Village.

• The raw water in the 3 Municipal Wells have tested at ranges between 150ng/L to 662ng/L of PFOA. Contamination was from a Plastics Fabrication Company located in the village.
Hoosick Falls
Installation of Carbon Filtration System

GAC Filter reduced the PFOA Level to <2 ng/L in Public Water supply

EPA health advisory set at 70ng/L for $\Sigma$(PFOA+PFOS)
Public Health Lab Role

Exposure Assessment

Biomonitoring

Blood Collection
LRN-C and High Throughput Capability

- Serum Testing offered to all Hoosick Falls Residents (3,500 village + 3,100 town - using private wells).
- Requested to leverage LRN-C expertise and surge capacity to develop rapid, CLIA compliant biomonitoring capability for PFOA.
- 2,081 serum samples were analyzed in a ~8 weeks.
- Method for 11 PFAS targets was developed in parallel and also validated to CLIA standards for emerging issues at other NY and States water supplies.
- Projected workload would increase and data processing and sample collection required alternative solutions.
Serum PFAS Analysis

Sample Pretreatment

Specimen matrix clean up Phenomenex Phree 96 Well Plate (protein/ phospholipid removal)

- Perkin-Elmer Janus Automated liquid handler
- Dispense 500 µL acetonitrile internal standard solution into each well
- Transfer 50 µL of room temperature specimens/calibrators/controls into appropriate wells
- Vortex Phree plate for 2 min and place on a vacuum manifold, vacuum
- Collect filtrate Using Nunc 96 Deep well PP collection plate.
- Concentrate filtrate to approx. 150 µL volume using Genevac Vacuum Evaporator
- Transfer collection plate into UHPLC/MS/MS system for analysis

Quantitative analysis

Liquid Chromatography – MS/MS with isotope dilution quantitation.

- Agilent 1290 UHPLC system, Agilent 6460 triple quadrupole mass spectrometer equipped with electrospray ionization source.
- Run time: 6 min
- Flow rate: 0.3 mL/min
- Gradient: ACN/ 0.1% Ammonium Acetate
- Waters Acquity UPLC BEH C18 1.7 µm 2.1 x 50mm column or equivalent
- MRM scan with Negative polarity
- Throughput 160 Sample prepared for LC/MS/MS in 2 hrs
# 96 Well Plate Map

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<td>96</td>
</tr>
</tbody>
</table>

- **St**: Calibration Standard
- **QB**: Quality Control Serum Blank
- **CC**: Second Source Calibration Check Standard
- **QC**: Quality Control Check
- **#**: Human Serum Sample
Development of high throughput LC/MS/MS method for analysis of perfluorooctanoic acid from serum, suitable for large-scale human biomonitoring

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ABSTRACT

A simple method for determination of perfluorooctanoic acid (PFOA) from human serum by liquid chromatography/tandem mass spectrometry (LC/MS/MS) and utilizing high-throughput sample preparation was developed by New York State Public Health Emergency Preparedness Laboratory and used for biomonitoring studies. 50 μl of serum was mixed with a 0.4 ml of acetonitrile containing isotopically labeled internal standard, followed by phospholipid removal/protein precipitation. The extract was partially dried and analyzed by LC/MS/MS. The linear range of PFOA analysis was 0.5–100 ng/ml. LLOQ was selected as 0.5 ng/ml. The method was validated following APHL guidelines for LRN-C laboratories and about 6000 specimens were successfully prepared and analyzed using this simple LC/MS method. Due to sample preparation simplicity, the presented method can be used in large-scale clinical testing, such as public health surveillance studies.

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Serum Testing sites for PFAS

NY  3
VT  1
NH  1
PFAS Contamination

• **Newburg’s** public water supply is taken from Lake Washington and served 28,000 residents.
  This lake is impacted by run off from the Air National Guard AFB where Aqueous Film-forming Firefighting Foam has been used (AFFF).

• **Petersburgh’s** Wells were contaminated by a Plastics manufacturing plant.

• NH and VT samples were collected and shipped directly to Wadsworth by each state public health lab.
Drinking Water PFAS Profiles

Newburgh impacted mainly by AFFF contamination
Newburgh NY on the Hudson River
Results of Serum Analysis
Data Summary of Blood Test Results for People Tested February–April 2016

<table>
<thead>
<tr>
<th># of Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (including children)</td>
</tr>
<tr>
<td>Total adults (age 18 and over)</td>
</tr>
<tr>
<td>Adults who drank Village water</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Totals by gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
</tr>
<tr>
<td>Males</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Totals by age group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-17</td>
</tr>
<tr>
<td>18-39</td>
</tr>
<tr>
<td>40-59</td>
</tr>
<tr>
<td>60 and older</td>
</tr>
</tbody>
</table>

Hoosick Falls Samples
PFOA Blood Levels of Hoosick Residents Compared to Residents from Other Communities

PFOA level in Blood:
Hoosick 50th Percentile Compared to US Population and Other Community Studies of Public Water Supplies
(Micrograms per Liter)

- Little Hocking, OH: 228
- Lubeck, WV: 92
- People on Hoosick Falls Public Water: 64.2
- Tumpers Plains, OH: 42
- Total Hoosick Falls area: 28.3
- Mason County, WV: 16
- US Population: 2.1
Adults using village water – levels are 50th percentiles
Data Summary for Children on By Age Group and Drinking Water Source

<table>
<thead>
<tr>
<th>By Age Groups</th>
<th># of Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>All children</td>
<td>353</td>
</tr>
<tr>
<td>Younger than age 12</td>
<td>199</td>
</tr>
<tr>
<td>Ages 12-17</td>
<td>154</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>By Drinking Water Source</th>
<th># of Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Currently served by Village public water</td>
<td>212</td>
</tr>
<tr>
<td>Formerly served by Village public water</td>
<td>55</td>
</tr>
<tr>
<td>Never served by Village public water</td>
<td>86</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Currently Served by Village Public Water by Age Group</th>
<th># of Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Younger than age 6</td>
<td>38</td>
</tr>
<tr>
<td>Ages 6 to 10</td>
<td>64</td>
</tr>
<tr>
<td>Ages 11 to 17</td>
<td>110</td>
</tr>
</tbody>
</table>

PFOA Blood Test Results for All Children by Drinking Water Source (Micrograms per Liter)

- Currently served by Hoosick Falls public water: 33.8
- Formerly served by Hoosick Falls public water: 12
- Never served by Hoosick Falls public water: 5.2
Recently Reported Serum Test Results for PFAS

<table>
<thead>
<tr>
<th>State/Town</th>
<th>Samples tested</th>
<th>Target Analytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Hampshire PHL</td>
<td>594</td>
<td>N-MeFOSAA, PFBuS, PFDeA, PFDooA, PFHpA, PFHxS, PFNA, PFOA, PFOS, PFOSA, PFUA (11 analytes)</td>
</tr>
<tr>
<td>Newburgh</td>
<td>1,566</td>
<td>PFBuS, PFHpA, PFHxS, PFNA, PFOA, PFOS (6 analytes)</td>
</tr>
<tr>
<td>Hoosick Falls</td>
<td>1,018</td>
<td>PFOA</td>
</tr>
<tr>
<td></td>
<td>2,081*</td>
<td></td>
</tr>
<tr>
<td>Vermont PHL</td>
<td>82</td>
<td>PFOA</td>
</tr>
<tr>
<td>Petersburgh</td>
<td>156</td>
<td>PFOA</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>5,497</strong></td>
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</table>

*Analyzed Feb-April 2016
### Serum Testing for PFAS (ug/L)

<table>
<thead>
<tr>
<th>LOCATION</th>
<th># Samples</th>
<th>PFOA</th>
<th>PFOS</th>
<th>PFHxS</th>
<th>PFNA</th>
<th>PFBuS</th>
<th>PFHpA</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Hampshire</td>
<td>566</td>
<td>5.17</td>
<td>9.34</td>
<td>4.09</td>
<td>0.85</td>
<td>0.502</td>
<td>0.550</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>5.17</td>
<td>9.34</td>
<td>4.09</td>
<td>0.85</td>
<td>0.502</td>
<td>0.550</td>
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<tr>
<td>50th Percentile</td>
<td></td>
<td>3.17</td>
<td>6.72</td>
<td>1.69</td>
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<td>0.500</td>
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<tr>
<td>95th Percentile</td>
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<td>13.7</td>
<td>1.69</td>
<td>0.500</td>
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<tr>
<td>Newburgh</td>
<td>1566</td>
<td>3.14</td>
<td>19.8</td>
<td>12.6</td>
<td>0.934</td>
<td>0.503</td>
<td>0.507</td>
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<tr>
<td>Average</td>
<td></td>
<td>3.14</td>
<td>19.8</td>
<td>12.6</td>
<td>0.934</td>
<td>0.503</td>
<td>0.507</td>
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<tr>
<td>50th Percentile</td>
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<td>2.37</td>
<td>11.8</td>
<td>6.40</td>
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<tr>
<td>95th Percentile</td>
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<td>61.8</td>
<td>44.3</td>
<td>1.94</td>
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<td>Vermont</td>
<td>82</td>
<td>12.0</td>
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<td>50th Percentile</td>
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<td>3.22</td>
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<tr>
<td>95th Percentile</td>
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<td>34.8</td>
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<tr>
<td>Hoosick Falls/Petersburgh</td>
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<td>95th Percentile</td>
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<td>177</td>
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Serum Profiles (ug/L)

Newburgh
N=1566

New Hampshire
N=566
Importance of Remote Order Entry

- Demographic data entry at collection sites and electronic data transfer (ROE) to the Wadsworth Center.

- This process eliminates; 1) manual data entry at Wadsworth Center or CEH database upload, and 2) discrepancies between the specimen label and the test requisition
  - Require secure file transfer to resolve (personal identifiable information (PII))

- Electronic data transfer (ROE) ensures that all samples received have existing demographic data entered into our system and a test referral request (test requisition) from the collection facility in place

- In place for emergency response for hospitals and clinics that are part of the NYS DOH Health Commerce System when completed.
Ongoing Work

• Continue monitoring of Drinking Water
  – Effectiveness of Carbon Filtration
• Serum Testing for impacted locations
• Repeat serum testing to document effects of remediation and reduced exposure.
• Identify other priority PWS emerging contaminants.
• Report on each project findings
Thank You

Contact Information
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Phone: (518) 474-7161