United States
Environmental Protection
Agency

Method Development for Unregulated Contaminants in Drinking Water: Public Meeting and Webinar

Held June 6, 2018
USEPA, Office of Ground Water and Drinking Water

Office of Water (MLK 140) EPA 815-A-18-001 June 2018

Methods Development for Unregulated Contaminants in Drinking Water

Public Meeting and Webinar

June 6, 2018

9:00 a.m. - 3:00 p.m. ET

U.S. EPA

Office of Water and

Office of Research and Development

Welcome
&
SDWA Regulatory Process

Brenda Parris, U.S. EPA

Office of Ground Water and Drinking Water

Technical Support Center

Participating by Webinar

- Listen-only mode
- Click on "+" next to "Questions" in the control panel (Figure 1) to submit questions/comments
  - Type a question in the box; click send (Figure 2)
- Submit questions as soon as possible
  - Questions will be answered at the end of the presentations

Figure 1

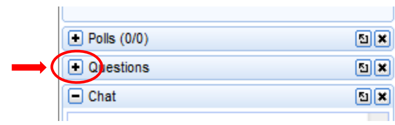
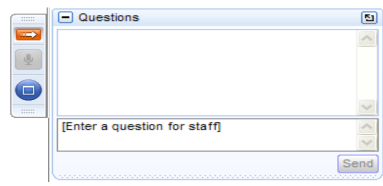


Figure 2



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Agenda	
8:30-9:00	Stakeholder Sign-In
	Welcome & SDWA Regulatory Process
	Overview of Method Development
	EPA Method 542
	EPA Methods 524.2/524.3/524.4 and 525.3
	EPA Method 556.1
~10:15-10:30	Break
	EPA Method 540 & 543
	EPA Methods 537 & 538
	Method in Development: PFAS
	Method in Development 558: Ethyl carbamate (Urethane) and N-Methyl-2-pyrrolidone
	Method in Development: Nonylphenols
~11:45-12:45	Lunch
	Method in Development: Legionella
	Method in Development: Mycobacterium
~1:45-2:00	Break
2:00-3:00	Open Forum and Discussion
	Closing Remarks

Overview

- Regulatory background for UCMR
  - Safe Drinking Water Act (SDWA) authority
  - Relationships to:
    - Contaminant Candidate List (CCL)
    - Unregulated Contaminant Monitoring Rule (UCMR)
    - Regulatory Determination
    - Six-Year Review

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SDWA

- Enacted in 1974, SDWA authorized EPA to set enforceable health standards for contaminants in drinking water
  - National Primary Drinking Water Regulations (NPDWRs)
- 1986 SDWA amendments were the basis for the original UCMR
  - State drinking water programs managed the original UCM program
  - PWSs serving > 500 people were required to monitor
- 1996 SDWA amendments changed the process of developing and reviewing NPDWRs
  - CCL
  - UCMR
  - Regulatory Determination
  - Six-Year Review

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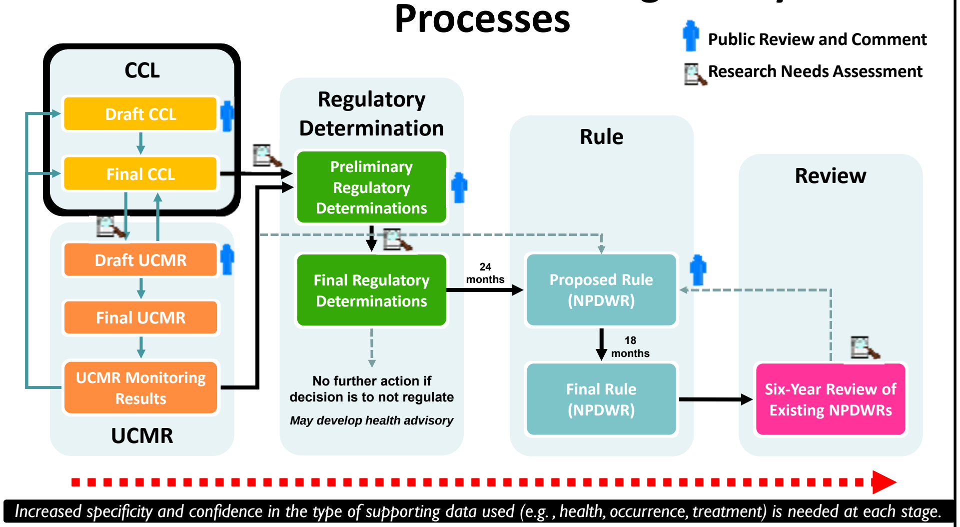
General Flow of SDWA Regulatory Processes



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CCL

- SDWA 1412(b)(1)(B) established listing of contaminants for regulatory consideration:
  - EPA shall publish a list of contaminants which are:
    - not subject to any proposed or promulgated NPDWR, which are known or anticipated to occur in PWSs, and
    - which may require regulation under SDWA

The Final CCL 4 was published November 17, 2016 and included 97 chemicals or chemical groups and 12 microbes

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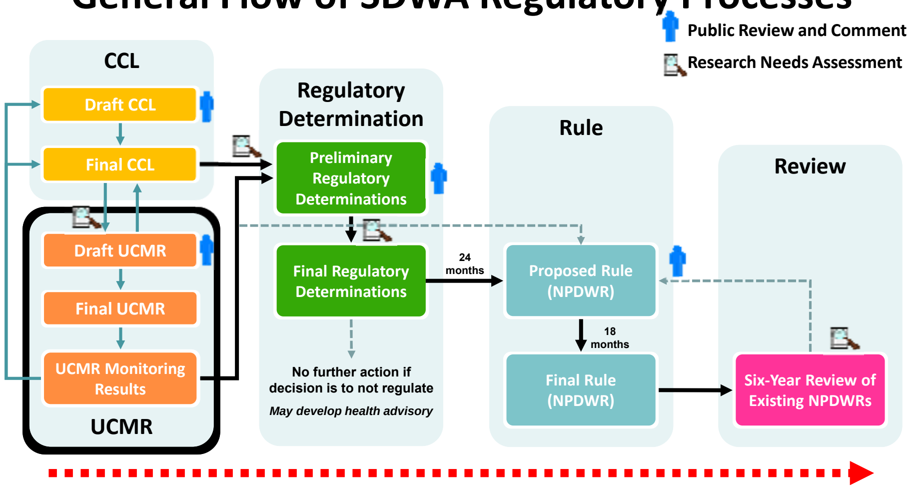
CCL 4

1,1,1,2-Tetrachloroethane (502.2, 524.2, 524.3, 524.4)	Erythromycin (542)	Oxirane, methyl-
1,1-Dichloroethane (UCMR 3)	Estradiol (17-beta estradiol) (UCMR 3)	Oxydemeton-methyl (538)
1,2,3-Trichloropropane (UCMR 3)	Estriol (UCMR 3)	Oxyfluorfen (UCMR 4)
1,3-Butadiene (UCMR 3)	Estrone (UCMR 3)	Perfluorooctane sulfonic acid (PFOS) (UCMR 3)
1,4-Dioxane (UCMR 3)	Ethinyl Estradiol (17-alpha ethynyl estradiol) (UCMR 3)	Perfluorooctanoic acid (PFOA) (UCMR 3)
17 alpha-Estradiol	Ethoprop (UCMR 4)	Permethrin (UCMR 4)
1-Butanol (UCMR 4)	Ethylene glycol	Profenofos (UCMR 4)
2-Methoxyethanol (UCMR 4)	Ethylene Oxide	Quinoline (UCMR 4)
2-Propen-1-ol (UCMR 4)	Ethylene thiourea	RDX (UCMR 2)
3-Hydroxycarbofuran (531.1, 531.2, 540, 543)	Formaldehyde (556, 556.1)	sec-Butylbenzene (502.2, 524.2, 524.3, 524.4)
4,4'-Methylenedianiline	Germanium (UCMR 4)	Tebuconazole (UCMR 4)
Acephate (538)	Halon 1011 (bromochloromethane) (UCMR 3)	Tebufenozide (540, 543)
Acetaldehyde (556, 556.1)	HCFC–22 (UCMR 3)	Tellurium
Acetamide	Hexane	Thiodicarb
Acetochlor (UCMR 1, UCMR 2)	Hydrazine	Thiophanate-methyl
Acetochlor ethanesulfonic acid (ESA) (UCMR 2)	Manganese (UCMR 4)	Toluene diisocyanate
Acetochlor oxanilic acid (OA) (UCMR 2)	Mestranol	Tribufos (UCMR 4)
Acrolein	Methamidophos (538)	Triethylamine
Alachlor ethanesulfonic acid (ESA) (UCMR 2)	Methanol	Triphenyltin hydroxide (TPTH)
Alachlor oxanilic acid (OA) (UCMR 2)	Methyl bromide (Bromomethane) (UCMR 3)	Urethane (In Development)
alpha-Hexachlorocyclohexane (UCMR 4)	Methyl tert-butyl ether (UCMR 1)	Vanadium (UCMR 3)
Aniline	Metolachlor (UCMR 2)	Vinclozolin (525.3, 527)
Bensulide (540, 543)	Metolachlor ethanesulfonic acid (ESA) (UCMR 2)	Ziram
Benzyl chloride	Metolachlor oxanilic acid (OA) (UCMR 2)	Adenovirus
Butylated hydroxyanisole (UCMR 4)	Molybdenum (UCMR 3)	Caliciviruses (UCMR 3)
Captan	Nitrobenzene (UCMR 1)	Campylobacter jejuni
Chlorate (UCMR 3)	Nitroglycerin	Enterovirus (UCMR 3)
Chloromethane (Methyl chloride) (UCMR 3)	N-Methyl-2-pyrrolidone (In Development)	Escherichia coli (O157)
Clethodim	N-Nitrosodiethylamine (NDEA) (UCMR 2)	Helicobacter pylori
Cobalt (UCMR 3)	N-Nitrosodimethylamine (NDMA) (UCMR 2)	Hepatitis A virus
Cumene hydroperoxide	N-Nitroso-di-n-propylamine (NDPA) (UCMR 2)	Legionella pneumophila (In Development)
Cyanotoxins (UCMR 4)	N-Nitrosodiphenylamine	Mycobacterium avium (In Development)
Dicrotophos (538)	N-Nitrosopyrrolidine (NPYR) (UCMR 2)	Naegleria fowleri
Dimethipin (UCMR 4)	Nonylphenol (In Development)	Salmonella enterica
Diuron (UCMR 1)	Norethindrone (19-Norethisterone)	Shigella sonnei
Equilenin	n-Propylbenzene (502.2, 524.2, 524.3, 524.4)	
Equilin (UCMR 3)	o-Toluidine (UCMR 4)	

General Flow of SDWA Regulatory Processes



Increased specificity and confidence in the type of supporting data used (e.g., health, occurrence, treatment) is needed at each stage.

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UCMR

- SDWA section 1445(a)(2), established requirements for the UCMR Program:
  - Issue list of no more than 30 unregulated contaminants, once every 5 years
  - Require PWSs serving population >10,000 people as well as a nationally representative sample of PWSs serving ≤10,000 people to monitor
  - Store analytical results in the National Contaminant Occurrence Database (NCOD) for Drinking Water
  - EPA funds shipping/analytical costs for small PWSs
- EPA manages program in partnership with states

UCMR History

- UCMR 1 (2001-2005, 26 contaminants)
- UCMR 2 (2007-2011, 25 contaminants)
- UCMR 3 (2012-2016, 30 contaminants)
- UCMR 4 (2017-2021, 30 contaminants)
  - Published in the FR on December 20, 2016
  - PWSs monitor 2018-2020

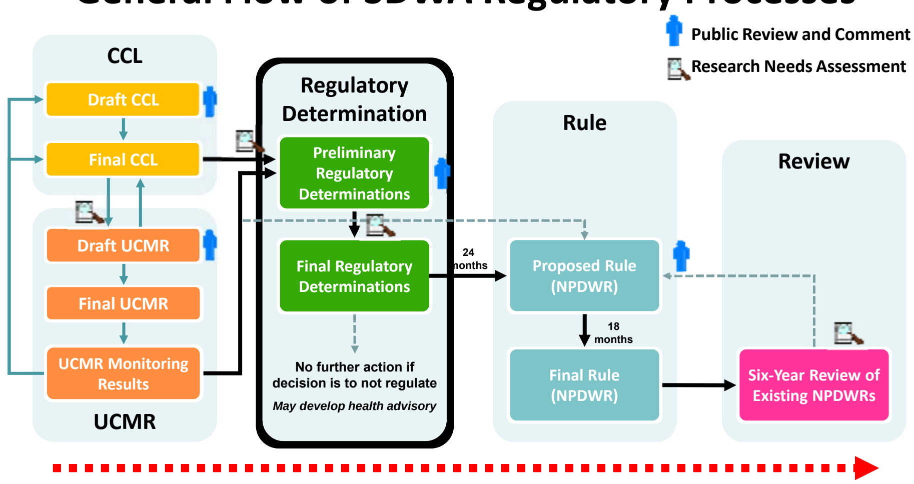
Each new UCMR cycle is established via a revision to the rule for the ongoing/preceding cycle.

Objective of UCMR Program

- Collect nationally representative occurrence data for unregulated contaminants that may require regulation under the SDWA
    - Consider data collected as part of future EPA decisions on actions to protect public health
    - Provide data to States, local governments and to the public for their use in decisions regarding public health protection

National occurrence data publically available:
http://www.epa.gov/dwucmr/occurrence-data-unregulated-contaminant-monitoring-rule

General Flow of SDWA Regulatory Processes



Increased specificity and confidence in the type of supporting data used (e.g., health, occurrence, treatment) is needed at each stage.

Regulatory Determinations

- Every five years, the Administrator shall, after notice of the preliminary determination and opportunity for public comment, for not fewer than five contaminants included on the CCL, make determinations on whether or not to regulate such contaminants.
- SDWA requires EPA to publish a maximum contaminant level goal (MCLG) and promulgate an NPDWR for a contaminant if the Administrator determines that:

1. The contaminant may have an **adverse effect** on the health of persons;
2. The contaminant is **known to occur or there is substantial likelihood** that the contaminant will occur in public water systems with a frequency and at levels of public health concern; **and**
3. In the sole judgment of the Administrator, regulation of such contaminant presents a meaningful opportunity for health risk reduction for persons served by public water systems.

**SDWA Section 1412(b)(1)*

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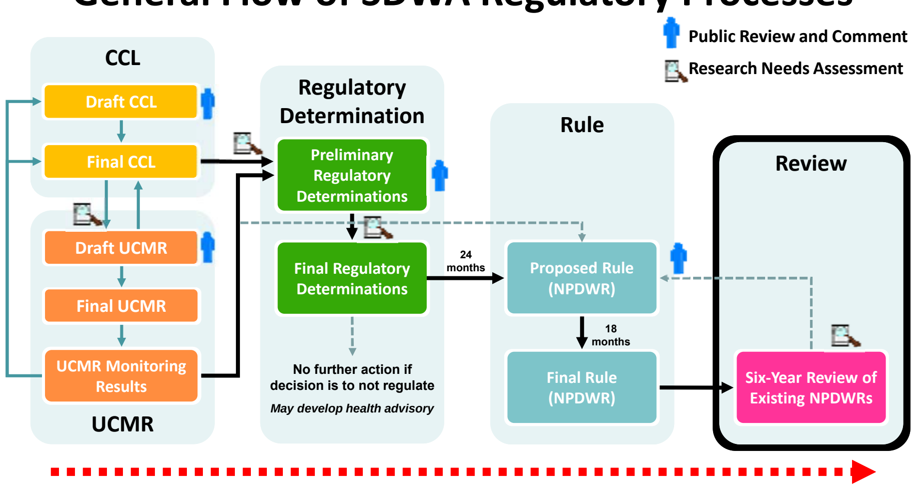
General Flow of SDWA Regulatory Processes



Increased specificity and confidence in the type of supporting data used (e.g., health, occurrence, treatment) is needed at each stage.

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Six-Year Review

- Reviews existing NPDWRs, every six years, and determines if a revision is appropriate
  - Includes the re-evaluation of new information on health effects, treatment technologies, analytical methods, occurrence and exposure, implementation and/or other factors that provide a health or technical basis to support a regulatory revision that will improve public health protection.
- Any revisions to existing NPDWRs must maintain protection or provide for greater health protection

**SDWA Section 1412(b)(9)*

CCL 4 Contaminants Monitored in UCMRs

1,1-Dichloroethane (UCMR 3)	Dimethipin (UCMR 4)	Nitrobenzene (UCMR 1)
1,2,3-Trichloropropane (UCMR 3)	Diuron (UCMR 1)	N-Nitrosodiethylamine (NDEA) (UCMR 2)
1,3-Butadiene (UCMR 3)	Equilin (UCMR 3)	N-Nitrosodimethylamine (NDMA) (UCMR 2)
1,4-Dioxane (UCMR 3)	Estradiol (17-beta estradiol) (UCMR 3)	N-Nitroso-di-n-propylamine (NDPA) (UCMR 2)
1-Butanol (UCMR 4)	Estriol (UCMR 3)	N-Nitrosopyrrolidine (NPYR) (UCMR 2)
2-Methoxyethanol (UCMR 4)	Estrone (UCMR 3)	o-Toluidine (UCMR 4)
2-Propen-1-ol (UCMR 4)	Ethinyl Estradiol (17-alpha ethynyl estradiol) (UCMR 3)	Oxyfluorfen (UCMR 4)
Acetochlor (UCMR 1, UCMR 2)	Ethoprop (UCMR 4)	Perfluorooctane sulfonic acid (PFOS) (UCMR 3)
Acetochlor ethanesulfonic acid (ESA) (UCMR 2)	Germanium (UCMR 4)	Perfluorooctanoic acid (PFOA) (UCMR 3)
Acetochlor oxanilic acid (OA) (UCMR 2)	Halon 1011 (bromochloromethane) (UCMR 3)	Permethrin (UCMR 4)
Alachlor ethanesulfonic acid (ESA) (UCMR 2)	HCFC–22 (UCMR 3)	Profenofos (UCMR 4)
Alachlor oxanilic acid (OA) (UCMR 2)	Manganese (UCMR 4)	Quinoline (UCMR 4)
alpha-Hexachlorocyclohexane (UCMR 4)	Methyl bromide (Bromomethane) (UCMR 3)	RDX (UCMR 2)
Butylated hydroxyanisole (UCMR 4)	Methyl tert-butyl ether (UCMR 1)	Tebuconazole (UCMR 4)
Chlorate (UCMR 3)	Metolachlor (UCMR 2)	Tribufos (UCMR 4)
Chloromethane (Methyl chloride) (UCMR 3)	Metolachlor ethanesulfonic acid (ESA) (UCMR 2)	Vanadium (UCMR 3)
Cobalt (UCMR 3)	Metolachlor oxanilic acid (OA) (UCMR 2)	Caliciviruses (UCMR 3)
Cyanotoxins (UCMR 4)	Molybdenum (UCMR 3)	Enterovirus (UCMR 3)

CCL 4 Contaminants Not Yet Monitored in UCMR

Method Available	Method in Development	No Method or Current Development Activity by EPA	
1,1,1,2-Tetrachloroethane (502.2, 524.2, 524.3, 524.4)	N-Methyl-2-pyrrolidone	17 alpha-Estradiol	Adenovirus
3-Hydroxycarbofuran (531.1, 531.2, 540, 543)	Nonylphenol	4,4'-Methylenedianiline	Campylobacter jejuni
Acephate (538)	Urethane	Acetamide	Escherichia coli (O157)
Acetaldehyde (556, 556.1)	Legionella pneumophila	Acrolein	Helicobacter pylori
Bensulide (540, 543)	Mycobacterium avium	Aniline	Hepatitis A virus
Dicrotophos (538)		Clethodim	Naegleria fowleri
Erythromycin (542)		Equilenin	Salmonella enterica
Formaldehyde (556, 556.1)		Ethylene glycol	Shigella sonnei
Methamidophos (538)		Ethylene thiourea	
n-Propylbenzene (502.2, 524.2, 524.3, 524.4)		Hydrazine	
Oxydemeton-methyl (538)		Nitroglycerin	
sec-Butylbenzene (502.2, 524.2, 524.3, 524.4)		N-Nitrosodiphenylamine	
Tebufenozide (540, 543)		Norethindrone (19-Norethisterone)	
Vinclozolin (525.3, 527)		Oxirane, methyl-	
		Tellurium	
		Thiodicarb	
		Thiophanate-methyl	
		Triethylamine	
		Triphenyltin hydroxide (TPTH)	
		Ziram	
		Benzyl chloride*	
		Captan*	
		Cumene hydroperoxide*	
		Ethylene Oxide*	
		Hexane*	
		Mestranol*	
		Methanol*	
		Toluene diisocyanate*	

* Method Challenges

Questions

General Guidelines Used in U.S. EPA Drinking Water Method Development and Application

William A. Adams, Ph.D.

U.S. EPA
Office of Ground Water and Drinking Water
Technical Support Center

Overview

- General Method Development Process
- EPA Method 545 as example of approach

Drinking Water Method Attributes

- Preservation
  - Dechlorination
  - Storage Stability/Hold Time Studies
- Quality Control
- Quantitation Levels

Method Development Considerations

- Simplicity
  - No overly complicated steps
  - Relatively non-hazardous components
  - Ease of sample collection
  - Reasonable instrumentation
- Data Quality
  - Focus on QC to ensure valid data especially for potentially regulated contaminants

Laboratory Quality Control

- Initial Demonstration of Capability (IDC)
  - Demonstration of Low System Background
  - Precision and Accuracy
  - Minimum Reporting Level (MRL) Confirmation
  - Quality Control Sample (QCS) from Second Source
- Ongoing QC
  - Initial Calibration
  - Continuing Calibration Check (CCC)
  - Laboratory Reagent Blank (LRB)
  - Laboratory Fortified Blank (LFB)
  - Internal Standards (IS)
  - Surrogates Standards (SUR)
  - Laboratory Fortified Sample Matrix and Duplicates (LFSM, LFSMD)
  - QCS at intervals

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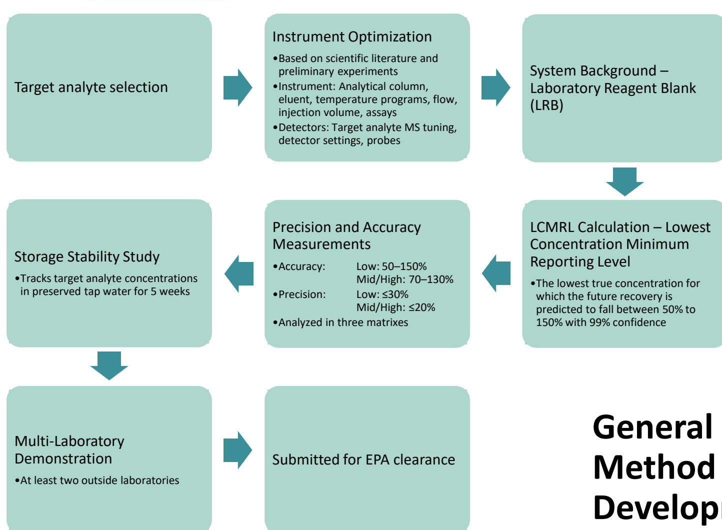

General Method Development

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Method Performance Data

- Evaluation of Method
  - Simplicity
  - Data Quality
- Demonstration of Low System Background using a Laboratory Reagent Blank (LRB)
- LCMRL
  - The lowest true concentration for which the future recovery is predicted to fall between 50% to 150% with 99% confidence
- Precision and Accuracy Study in Three Matrixes
  - Meet %Rec and %RSD thresholds
- Storage Stability Study
  - 35 Day study observing target analyte loss over time
- Second Laboratory Validation

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Laboratory Method Flexibility

Usually YES

- Instrumental conditions
  - Chromatography
  - Detector Parameters
  - Analytical Column
- Additional IS or SUR
- Different Manufacturers

Usually NO

- Sample Collection and Preservation
- Sample Preparation (e.g. Extraction, Elution)
- QC Requirements
- Prescribed IS or SUR
- Different Instrumentation

- Unless otherwise stated in the method
- Must verify method performance

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Method 545

Determination of Cylindrospermopsin and Anatoxin-a in Drinking Water by Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC/ESI-MS/MS)

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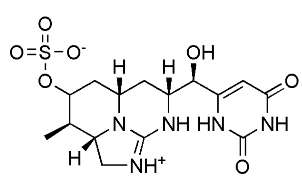
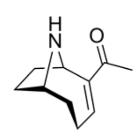
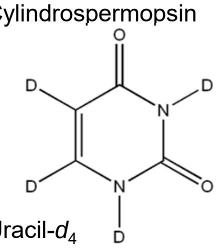
M545 Target Analytes and IS



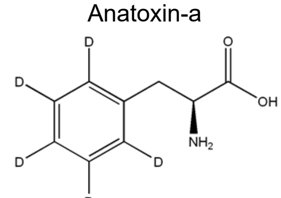
Cylindrospermopsin



Anatoxin-a



Uracil-d_4



L-phenylalanine-d_5

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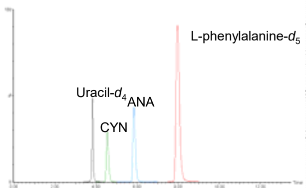
M545 Preservation

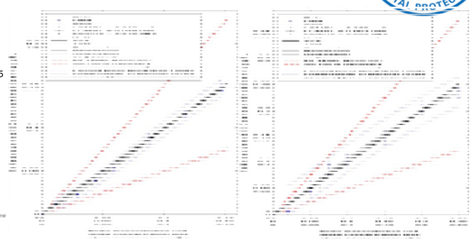
- Store samples in refrigerator (≤6 °C)
- 100 mg/L ascorbic acid
  - Reduces residual chlorine present in tap water samples
  - Easy to handle
  - Solid can be added to bottles before sampling
- 1000 mg/L sodium bisulfate
  - Acts as a microbial inhibitor
  - pH less than 3
  - Solid can be added to bottles before sampling
  - No observable interferences with direct injection

M545 Instrumental Method

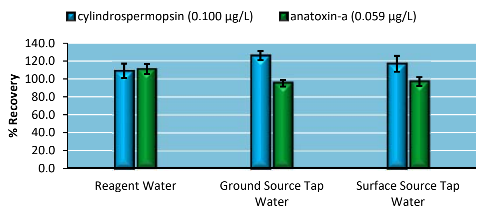
- Waters Acquity Liquid Chromatography (LC) / Quattro Premier XE triple quadrupole MS (ESI) [equivalents acceptable]
- Waters XSelect® HSS T3 2.1 x 150 mm, 3.5 µm analytical column, 30 °C [equivalents acceptable]
- 100 mM acetic acid in reagent water (A) and 100% methanol (B) step gradient at a 0.2 mL/min flow (Mostly isocratic @ 90% aqueous)
- 50-µL injections
- Ionization for all analytes was achieved through protonation ($[M+1]^+$)

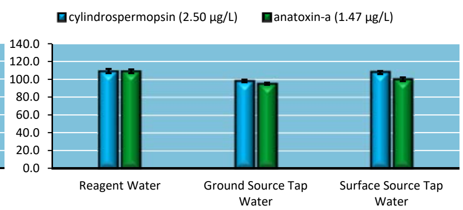
M545: Method Performance





Analyte	Fortified Conc. (µg/L)	Day 0		Day 7		Day 14		Day 21		Day 28	
		Avg. Meas. Conc. (µg/L)	%RSD	%Δ	%RSD	%Δ	%RSD	%Δ	%RSD	%Δ	%RSD
cylindrospermopsin	2.50	2.57	3.3	-5.4	2.4	-6.2	1.5	-5.1	1.2	-1.6	3.5
anatoxin-a	1.47	1.49	1.1	0.67	4.4	-7.4	2.4	-0.67	0.91	-1.3	2.1





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Questions

EPA Method 542: Analysis Of Erythromycin and Other Pharmaceuticals by LC-MS/MS

William A. Adams, Ph.D.

U.S. EPA
Office of Ground Water and Drinking Water, Technical Support Center

U.S. EPA Method 542

Method 542: Determination of Pharmaceuticals and Personal Care Products (PPCP) in Drinking Water by Solid Phase Extraction (SPE) and Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC/ESI-MS/MS)

September 2016, EPA 815-R-15-012

EPA Method 542 (LC-MS/MS)

Erythromycin	Gemfibrozil
Carbamazepine	Naproxen
Diazepam	Phenytoin
Diclofenac (sodium salt)	Sulfamethoxazole
Enalapril (maleate salt)	Triclosan
Fluoxetine (HCl)	Trimethoprim

Blue Fill: CCL 4 with methods; Plain: Included in method, not on CCL 4

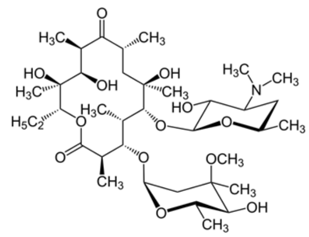
^{13}C-Naproxen-d_3, Triclosan-d_3, Carbamazepine-d_{10}, chosen as internal standards; ^{13}C-Trimethoprim-d_3 and Diclofenac-d_4 chosen as surrogate standards

Technology Overview

- Variety of chemically unrelated analytes
  - Both ESI positive and ESI negative modes using separate injections and elution programs
- Analysis by LC-MS/MS using a 5 mM ammonium acetate and methanol gradient
- SPE (6 cc, 200 mg HLB cartridge) followed by concentration step (100:1)
- Preservation
  - Refrigeration, 100 mg/L ascorbic acid, 350 mg/L Ethylenediaminetetraacetic acid (EDTA), 9.4.g/L potassium citrate
  - Solid preservatives can be added prior to sample collection

Erythromycin

- Antibiotic on CCL 4
- At pH <7, water is removed and compound no longer exhibits antibiotic properties (Hirsch et al., 1999)
- For analysis, erythromycin is measured as erythromycin–H_2O (717.0 > 158.3 *m/z*)



Erythromycin

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Method 542 Performance Data Highlights

- No significant blank interferences
- Precision and Accuracy Study in three tap water matrixes
  - Acceptable levels of matrix effects at low and middle concentrations
- Storage Stability Study
  - Sample and extract hold times change less than 20% after 28 days
- Second Laboratory Validation
  - External laboratories showed comparable results
- Sensitivity – LCMRL
  - The lowest true concentration for which the future recovery is predicted to fall between 50% to 150% with 99% confidence

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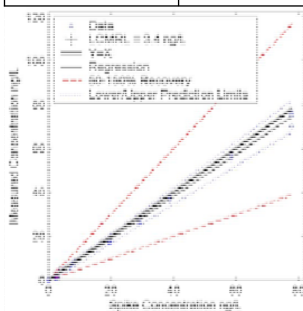
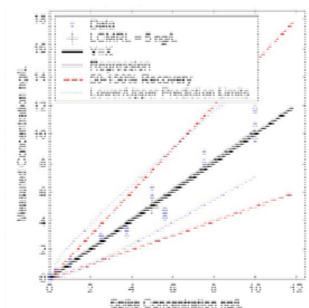
Analyte	LCMRL (ng/L)	Analyte	LCMRL (ng/L)
Carbamazepine	2.4	Naproxen	4.5
Diazepam	0.27	Phenytoin	1.4
Diclofenac	1.1	Sulfamethoxazole	0.28
Enalapril	0.60	**Triclosan**	**3.4**
Fluoxetine	0.98	Trimethoprim	4.1
Gemfibrozil	1.4	**Erythromycin**	**5.0**





EPA Method 542 LCMRL

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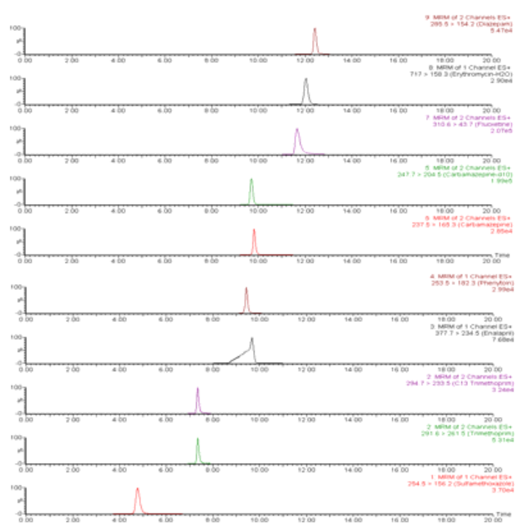
EPA Method 542: Pharmaceuticals and Personal Care Products



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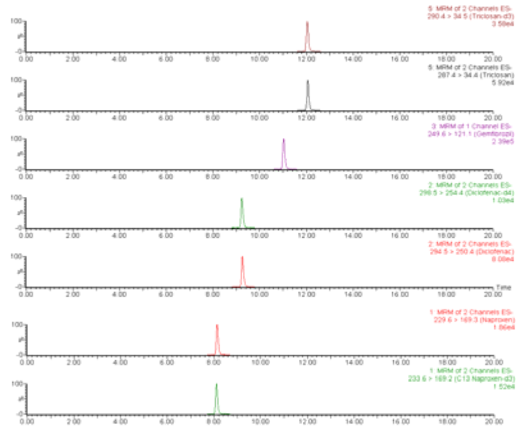
EPA Method 542: Pharmaceuticals and Personal Care Products



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Questions

Drinking Water Methods for Volatile and Semivolatile Compounds

Paul E. Grimmett
U.S. EPA
Office of Ground Water and Drinking Water
Technical Support Center

U.S. EPA Method 524.4

EPA Method 524.4: Measurement of Purgeable Organic Compounds in Water by Gas Chromatography / Mass Spectrometry using Nitrogen Purge Gas

May 2013, EPA 815-R-13-002

U.S. EPA Method 525.3

EPA Method 525.3: Determination of Semivolatile Organic Chemicals in Drinking Water by Solid Phase Extraction and Capillary Column Gas Chromatography /Mass Spectrometry (GC/MS)

February 2012, EPA 600-R-12-010

EPA Methods 524.2/524.3/524.4 (GC/MS)

1,1,1,2-tetrachloroethane	1,4-dichlorobenzene	1,1,2,2-tetrachloroethane	Diisopropyl ether (DIPE)
N-propylbenzene	Benzene	1,1-dichloropropene	Ethyl methacrylate
Sec-butylbenzene	Carbon tetrachloride	1,2,3-trichlorobenzene	Hexachlorobutadiene
1,1-dichloroethane	Bromodichloromethane	1,2,4-trimethylbenzene	Hexachloroethane
1,2,3-trichloropropane	Bromoform	1,2-dibromoethane	Isopropylbenzene
1,3-butadiene	Chlorobenzene	1,3,5-trimethylbenzene	Methyl acetate
Bromochloromethane (Halon 1011)	Chloroform	1,3-dichlorobenzene	Methyl iodide
Bromomethane (Methyl Bromide)	Cis-1,2-dichloroethene	1,3-dichloropropane	Naphthalene
Chlorodifluoromethane (HCFC-22)	Dibromochloromethane	1-chlorobutane	N-butylbenzene
Chloromethane (Methyl Chloride)	Ethylbenzene	2-chlorotoluene	Pentachloroethane
Methyl-t-butyl ether (MtBE)	Methylene chloride	4-chlorotoluene	T-amyl ethyl ether (TAEE)
1,1-dichloroethene	M-xylene	4-isopropyltoluene	T-amyl methyl ether (TAME)
1,1,1-trichloroethane	O-xylene	Allyl chloride	T-butyl alcohol (TBA)
1,1,2-trichloroethane	P-xylene	Bromobenzene	T-butyl ethyl ether (ETBE)
1,2,4-trichlorobenzene	Tetrachloroethene	Carbon disulfide	T-butylbenzene
1,2-dibromo-3-chloropropane (DBCP)	Trichloroethene	Cis-1,3-dichloropropene	Tetrahydrofuran
1,2-dichlorobenzene	Toluene	Dibromomethane	Trans-1,3-dichloropropene
1,2-dichloroethane	Trans-1,2-dichloroethene	Dichlorodifluoromethane	Trichlorofluoromethane
1,2-dichloropropane	Vinyl chloride	Diethyl ether	

Blue Fill: CCL 4; Green: Monitored under UCMR
Orange: Regulated; Plain: Included in method, not on CCL 4

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EPA Method 525.3 (GC/MS)

Vinclozolin	Terbacil
Chlorpyrifos	Alachlor (UCMR 2)
Dimethipin	Atrazine
Ethoprop	Benzo[a]pyrene
Hexachlorocyclohexane, alpha (α-HCH)	Chlordane (tech grade)
Oxyfluorfen	Di(2-ethylhexyl)adipate
Permethrin, cis-	Di(2-ethylhexyl)phthalate
Permethrin, trans-	Endrin
Profenofos	Heptachlor
Tebuconazole	Heptachlor epoxide
Tribufos	Hexachlorobenzene (HCB)
Acetochlor (UCMR 1)	Hexachlorocyclohexane, gamma (γ-HCH) (Lindane)
Metolachlor	Hexachlorocyclopentadiene (HCCPD)
DDE, 4,4'-	Methoxychlor
Dinitrotoluene, 2,4-	Pentachlorophenol
Dinitrotoluene, 2,6-	Simazine
Disulfoton	Toxaphene
EPTC (S-Ethyl dipropylthiocarbamate)	Polychlorinated Biphenyl (PCB) Congeners (IUPAC #)
Molinate	74 Additional Contaminants
Prometon	

Blue Fill: CCL 4; Green: Monitored under UCMR
Orange: Regulated; Plain: Included in method, not on CCL 4

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EPA Method 525.3 (GC/MS) Additional Contaminants

(chlordane) cis-chlordane	benzo (g,h,i) perylene	dimethyl phthalate	MGK 264
(chlordane) trans-chlordane	benzo (k) fluoranthene	di-n-butyl pthalate	napropamide
(chlordane) trans-nonachlor	bromacil	diphenamid	nitrofen
2,2′,3,4,4′,5,5′-heptachlorobiphenyl	butachlor	disulfoton	norflurazon
2,2′,3,4,4′,5′-hexachlorobiphenyl	butyl benzyl phthalate	endosufan I	pebulate
2,2′,3,4′,5′,6-hexachlorobiphenyl	butylate	endosufan II	phenanthrene
2,2′,3,5′-tetrachlorobiphenyl	chlorfenvinphos	endosufan sulfate	phorate
2,2′,5-trichlorobiphenyl	chlorobenzilate	ethion	phosphamidon
2,3,3′,4′,6-pentachlorobiphenyl	chloroneb	ethyl parathion	prometryn
2,3′,4,4′,5-pentachlorobiphenyl	chlorothalonil	etridiazole	pronamide
2,3′,4′,5-tetrachlorobiphenyl	chlorpropham	fenarimol	propachlor
2,4,4′-trichlorobiphenyl	chrysene	fluorene	propazine
2,4′-dichlorobiphenyl	cycloate	fluridone	pyrene
2-chlorobiphenyl	dacthal (DCPA)	hexachlorocyclohexane, beta	simetryn
4-chlorobiphenyl	DDD, 4,4'	hexachlorocyclohexane, delta	tebuthiuron
acenaphthylene	DDT, 4,4'	hexachlorocyclohexane, gamma	terbutryn
aldrin	DEET (N,N-diethyl-meta-toluamide)	hexazinone	tetrachlorvinphos
ametryn	di(2-ethylhexyl)phthalate	indeno [1,2,3-c,d]pyrene	triadimefon
anthracene	dibenz [a,h] anthracene	isophorone	trifluralin
atraton	dichlorvos	methyl parathion	vernolate
benzo (a) anthracene	dieldrin	metribuzin	
benzo (b) fluoranthene	diethyl phthalate	mevinphos	

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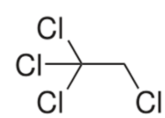
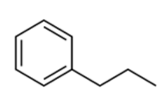
CCL 4 Compounds - VOCs
(yet to be monitored)

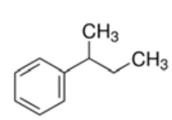
Volatile Organic Compounds



- 1,1,1,2-tetrachloroethane
  - solvent used in wood stains and varnishes
- n-propylbenzene
  - solvent used in printing and dying



- sec-butylbenzene
  - solvent used in surface coating



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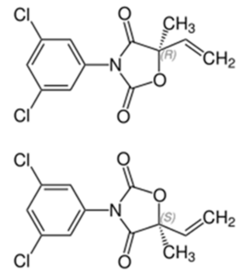
CCL 4 Compounds – Semivolatiles (yet to be monitored)

Semivolatile Organic Compounds

- vinclozolin
  - fungicide used on various fruits/vegetables



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Volatile Organic Compounds (VOCs)

Volatile Organic Compounds

- Normally high vapor pressures and low boiling points
- Natural or synthetic
- Form a gas easily (volatilize)
- Analysis commonly done by Gas Chromatography-Purge and Trap

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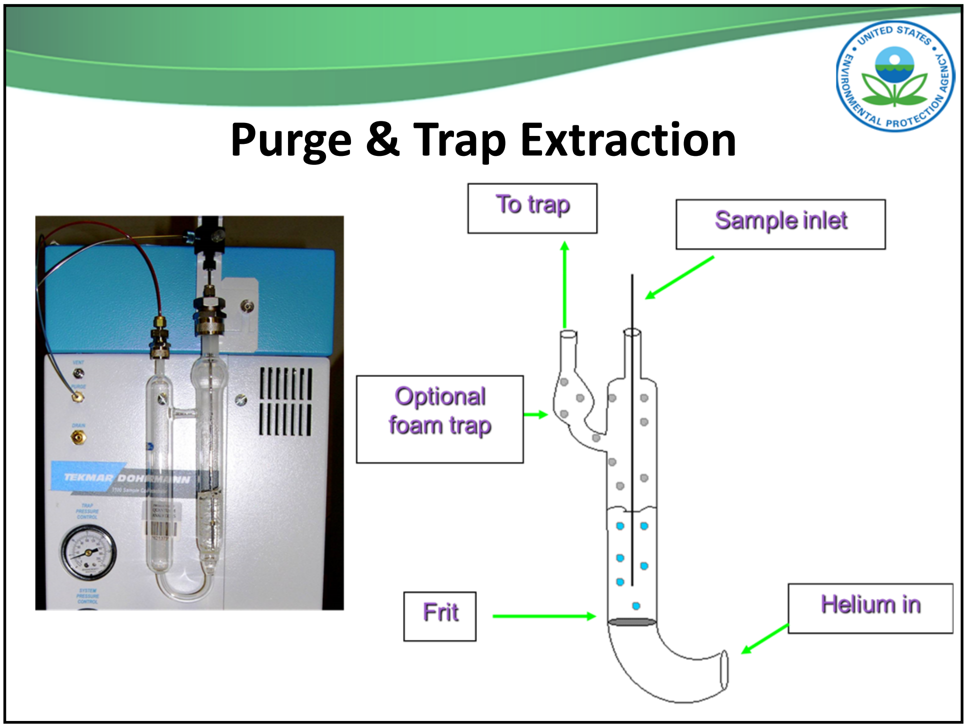
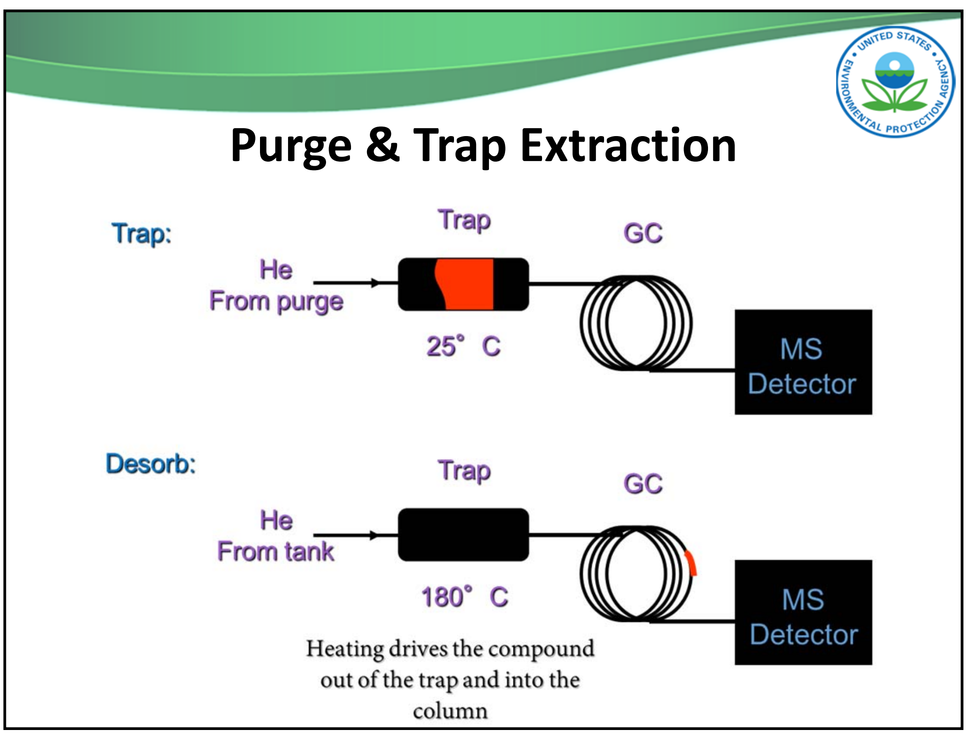

Purge & Trap Extraction
To trap
Sample inlet
Optional foam trap
Frit
Helium in


Purge & Trap Extraction
Trap:
Trap
GC
He
From purge
25° C
MS Detector
Desorb:
Trap
GC
He
From tank
180° C
MS Detector
Heating drives the compound out of the trap and into the column

Newer GC Methods for VOCs

Method 524.3 and Method 524.4

- GC/MS with purge and trap extraction
- Both approved through the expedited method approval process for monitoring regulated contaminants

*Federal Register / Vol. 74, No. 147 / Monday, August 3, 2009, p. 38348

*Federal Register / Vol. 78, No. 105 / Friday, May 31, 2013, p. 32558

- 1,1,1,2-tetrachloroethane, n-propylbenzene, and sec-butylbenzene are included in Methods 524.3 and 524.4

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Updates to Method 524

- Method 524.3 - allows more flexibility in purge & trap parameters

	Recommended		Allowable	
Parameter	Minimum	Maximum	Minimum	Maximum
Sample temperature	Ambient	40 °C	Ambient	60 °C
Purge flow rate	40 mL/min	80 mL/min	20 mL/min	200 mL/min
Purge volume	360 mL	520 mL	240 mL	680 mL
Desorb time	1 min	2 min	0.5 min	4 min
Purge volume + dry purge volume	360 mL	720 mL	240 mL	880 mL

- Method 524.4 – allows for purging with nitrogen instead of helium

	Recommended		Allowable	
Parameter	Minimum	Maximum	Minimum	Maximum
Sample temperature	Ambient	40 °C	Ambient	60 °C
Purge flow rate	40 mL/min	55 mL/min	20 mL/min	80 mL/min
Purge volume	360 mL	520 mL	320 mL	520 mL
Desorb time	1 min	1 min	0.5 min	2 min
Purge volume + dry purge volume	360 mL	720 mL	320 mL	720 mL

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Method 524 Comparison

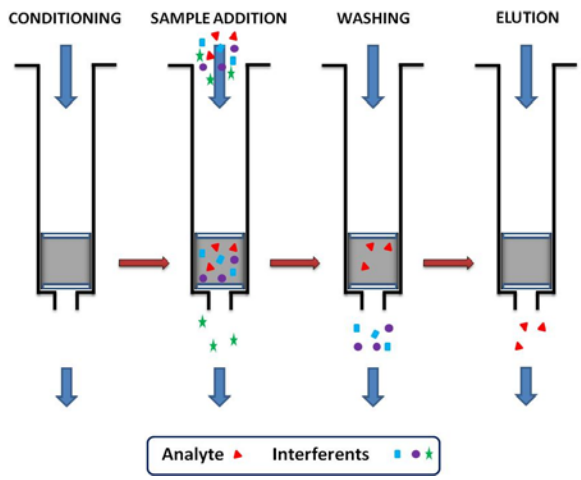
524.2	524.3	524.4
0.32 to 0.75 mm internal diameter (i.d.) columns	0.18 to 0.25 mm i.d. columns	0.18 to 0.25 mm i.d. columns
Cryogenic interface; no split; jet separator to MS	Split injection	Split injection
5 or 25-mL purge volume	5-mL purge volume	5-mL purge volume
Any trap that meets method criteria	Any trap that meets method criteria	Only traps containing synthetic carbon adsorbent media
Single concentrator	Single or tandem concentrator	Single or tandem concentrator
1 internal standard - fluorobenzene	3 internal standards - 1,4-difluorobenzene - chlorobenzene-d_5 - 1,4-dichlorobenzene-d_4	3 internal standards - 1,4-difluorobenzene - chlorobenzene-d_5 - 1,4-dichlorobenzene-d_4

Semivolatile Organic Compounds (SVOCs)

Semivolatile Organic Compounds

- Broad chemical properties and structural features
  - pesticides
  - flame retardants
  - PAHs, PCBs, etc.
- Natural or synthetic
- Higher boiling points and low vapor pressures
- Analysis commonly done by Gas Chromatography-Solid Phase Extraction (SPE), among others

Solid-Phase Extraction



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Solid-Phase Extraction

- Uses small volumes of solvent vs. traditional LLE / Sep Funnel methods
- Allows for large-scale concentration (better sensitivity)
- Less time consuming
- Minimal emulsion issues, i.e. no shaking

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Newer GC Method for SVOCs

Method 525.3

- SPE on polymeric sorbent (DVB), followed by GC/MS
- Approx. 130 analytes (pesticides, herbicides, PCBs, PAHs, etc)
- Published in February 2012, approved by expedited method approval process (Federal Register / Vol. 77, No. 125 / Thursday, June 28, 2012, p. 38523)
- Contains vinclozolin
- Currently using in UCMR 4 for monitoring 9 compounds

Method 525.3 Improvements

- Improved preservation scheme – citrate buffer vs. acid
- Updated surrogates and internal standards
- Addition of SIM option – better sensitivity
- New PCB screening procedure

Summary of Procedure (Method 525.3)

DVB disk or cartridge

Rinse, condition, wet: methylene chloride, ethyl acetate, methanol, and reagent water

Add surrogates to 1-L sample. → Add 1-L water sample, extract at ~10 mL/min. Rinse with reagent water.

Dry cartridge for 10 minutes.

Elute with ethyl acetate & methylene chloride.

Pass through Na_2SO_4, and concentrate extract to 1 mL. ← Add internal standards

Analyze extract by GC/MS

Conclusion

CCL 4 has a number of VOC and SVOC contaminants that have applicable EPA methods associated with them.

1) Method 524.3/524.4

Compound	MRL (µg/L)*	HRL (µg/L)**
1,1,1,2-tetrachloroethane	0.018	1
n-propylbenzene	0.030	5.83
sec-butylbenzene	0.035	10.3

2) Method 525.3

Compound	MRL (µg/L)*	HRL (µg/L)**
vinclozolin	0.028	0.549

* Multi-laboratory calculation, SIM

** Contaminant Information Sheets (CISs) for the Final Fourth Contaminant Candidate List - EPA 815-R-16-003

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Questions

EPA Method 556.1: Determination of Carbonyl Compounds in Drinking Water by Fast Gas Chromatography

Steve Wendelken, Ph.D.

U.S. EPA
Office of Ground Water and Drinking Water
Technical Support Center

U.S. EPA Method 556.1

EPA Method 556.1: Determination of Carbonyl Compounds in Drinking Water by Fast Gas Chromatography

September 1999

Method 556.1 Target Analyte List

Formaldehyde
Acetaldehyde
Propanal
Butanal
Pentanal
Hexanal
Heptanal
Octanal
Nonanal
Decanal
Cyclohexanone
Benzaldehyde
Glyoxal (ethanedial)
Methyl glyoxal (2-oxopropanal or pyruvic aldehyde)

Blue Fill: CCL 4 with methods; Plain: Included in method, not on CCL 4

556.1 Procedural Summary

- 20 mL sample adjusted to pH 4
- Derivitization (2h) with (2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBHA)
- Derivatized analytes extracted with 4 mL hexane
- Acid wash with 3 mL 0.2 N sulfuric acid
- Extracts are analyzed by gas chromatography with electron capture detection
- Some targets form 2 or more isomers whose peaks must be summed

Precision & Accuracy Finished Drinking Water (n=7)

Analyte	Fortified Concentration (mg/L)	Average Concentration (mg/L)	Unfortified Sample (mg/L)	Relative Standard Deviation	Average Recovery
Formaldehyde	5.0	8.45	3.40	3.3%	101%
Acetaldehyde	5.0	6.53	1.76	2.7%	96%
Propanal	5.0	5.68	0.620	2.2%	101%
Butanal	5.0	5.73	0.390	2.4%	107%
Pentanal	5.0	5.43	ND	2.6%	109%
Hexanal	5.0	5.48	ND	2.8%	110%
Cyclohexanone	5.0	6.02	0.650	4.2%	107%
Heptanal	5.0	5.64	0.840	4.1%	96%
Octanal	5.0	4.84	ND	6.4%	97%
Benzaldehyde	5.0	4.92	ND	3.1%	98%
Nonanal	5.0	5.25	0.250	8.5%	100%
Decanal	5.0	5.78	ND	8.9%	116%
Glyoxal	5.0	7.92	1.40	9.2%	130%
Methyl Glyoxal	5.0	6.42	0.380	9.2%	121%

Minimum Reporting Levels (MRLs)

- 556.1 was developed prior to the LCMRL process being instituted
- The MRLs for formaldehyde and acetaldehyde will be dependent on the laboratories' ability to control background levels of these analytes
- The most successful techniques for generating aldehyde free water are exposure to UV light, or distillation from permanganate
- A Millipore Elix 3 reverse osmosis system followed by a Milli-Q TOC Plus polishing unit provided a background < 1 μg/L

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Method Detection Limits

Analyte	Fortified Concentration (mg/L)	Primary Column MDL (mg/L)	Secondary Column MDL (mg/L)
Formaldehyde	1.0	0.09	0.08
Acetaldehyde	1.0	0.18	0.12
Propanal	1.0	0.11	0.06
Butanal	1.0	0.09	0.06
Pentanal	1.0	0.09	0.06
Hexanal	1.0	0.10	0.04
Cyclohexanone	1.0	0.19	0.09
Heptanal	1.0	0.40	0.24
Octanal	1.0	0.22	0.84
Benzaldehyde	1.0	0.19	0.04
Nonanal	1.0	0.62	0.64
Decanal	1.0	0.46	0.35
Glyoxal	1.0	0.39	0.13
Methyl Glyoxal	1.0	0.26	0.12

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Questions

Break

(15 minutes)

Drinking Water Methods Meeting/Webinar - 2018

Development of Method 538, 540, 543 and 537 for the Analysis of Chemicals on U.S. EPA's Contaminant Candidate List

Jody A. Shoemaker

Disclaimer: *Mention of trade names or commercial products does not constitute endorsement or recommendation for use.*

Office of Research and Development
National Exposure Research Laboratory

Method 538: DAI-LC/MS/MS (2009) Contains 11 Analytes

Method Analytes

acephate	aldicarb
dicrotophos	aldicarb sulfone
methamidophos	diisopropyl methylphosphonate (DIMP)
oxydemeton-methyl	fenamiphos sulfone
quinoline	fenamiphos sulfoxide
	thiofanox

Analytes in red are on CCL 4

- Most method analytes are pesticides (except for quinoline and DIMP) with the potential to contaminate drinking water sources
- Quinoline is an industrial starting material, a pharmaceutical (anti-malarial) and a flavoring agent
- DIMP is a chemical by-product in the production of sarin gas

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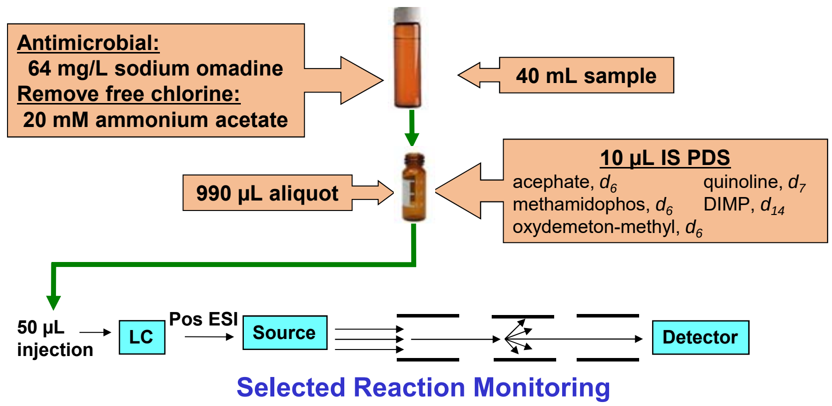
M538
Direct Aqueous Injection Approach



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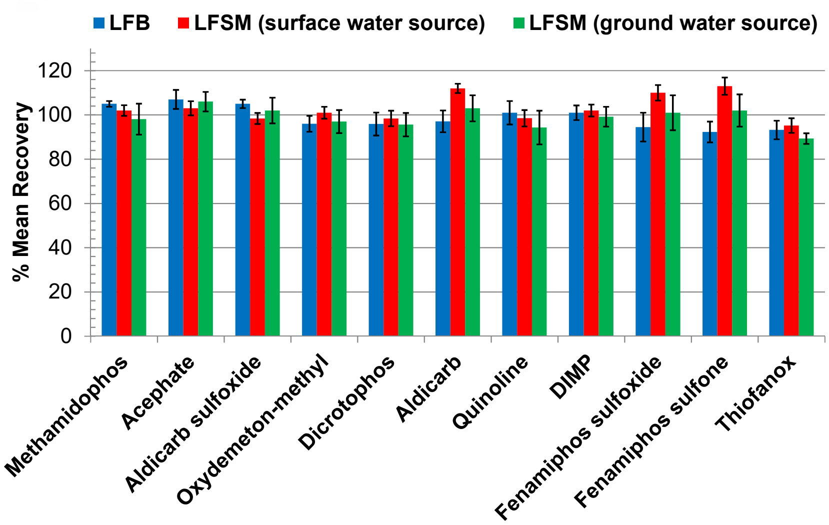
M538 Performance data (n=7)
LFBs and LFSMs fortified at 0.99 – 43 µg/L



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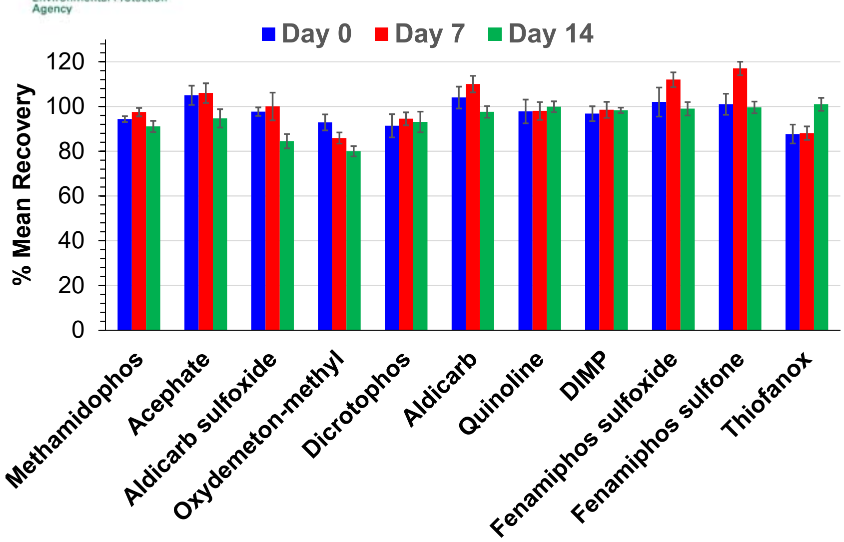
QC criteria at mid-level cal: 70-130%

EPA United States Environmental Protection Agency

M538 Aqueous Holding Time Data (n=7)



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EPA United States Environmental Protection Agency

M538 LCMRLs and HRLs

Analyte	LCMRL (ng/L)	HRL (ng/L)
Methamidophos	32	2100
Acephate	44	4000
Aldicarb sulfoxide	88	
Oxydemeton-methyl	19	910
Dicrotophos	39	490
Aldicarb	30	
Quinoline	1500*	10
DIMP	22	
Fenamiphos sulfoxide	42	
Fenamiphos sulfone	11	
Thiofanox	180	

LCMRL range: 11-180 ng/L

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EPA United States Environmental Protection Agency

Method 540: SPE-LC/MS/MS (2013) Contains 12 Analytes

Method Analytes

3-hydroxycarbofuran	fenamiphos sulfone
bensulide	fenamiphos sulfoxide
tebuconazole	methomyl
tebufenozide	chlorpryifos oxon
disulfoton sulfoxide	phorate sulfone
fenamiphos	phorate sulfoxide

Analytes in red are on CCL 4

- all method analytes are pesticides or pesticide degradates with the potential to contaminate drinking water sources

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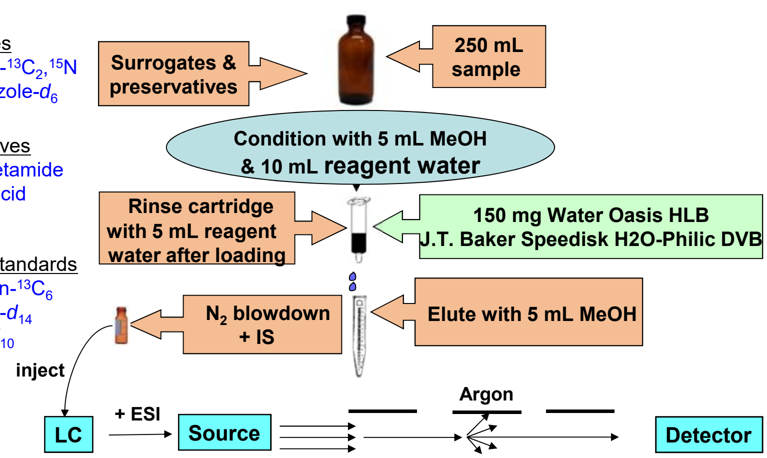
EPA United States Environmental Protection Agency

Method 540 Analytical Procedure

Surrogates
methomyl-$^{13}C_2$,^{15}N
tebuconazole-d_6

Preservatives
2-chloracetamide
ascorbic acid
Trizma

Internal Standards
carbofuran-$^{13}C_6$
bensulide-d_{14}
phorate-d_{10}



LC/MS/MS - Selected Reaction Monitoring

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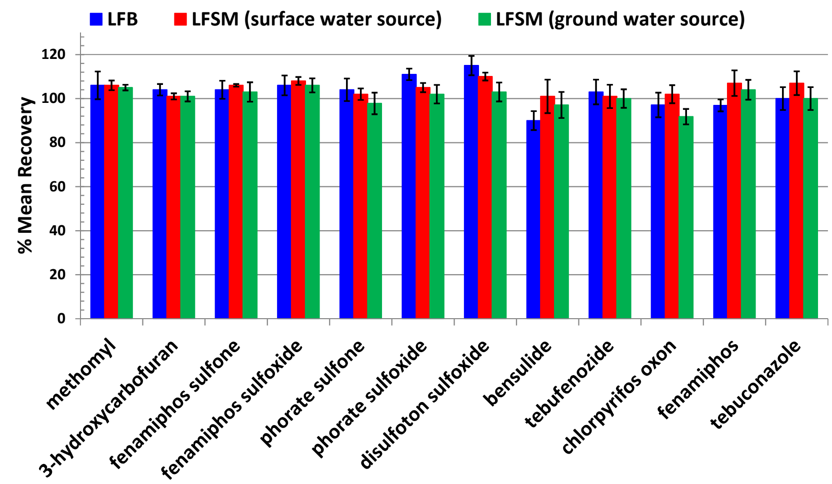
United States Environmental Protection Agency

M540 Performance Data

LFBs & LFSMs fortified at 12.8-32 ng/L (n=4)



QC criteria at mid-level cal: 70-130%

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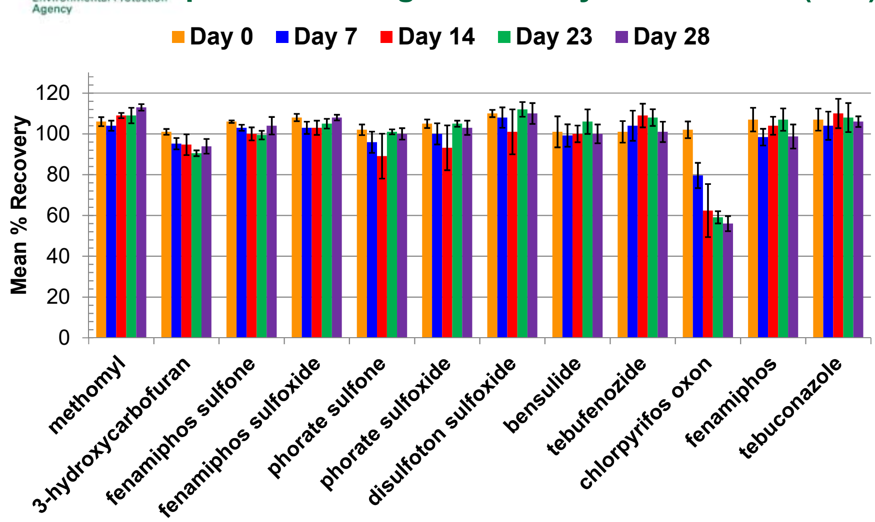
United States Environmental Protection Agency

Aqueous Holding Time Study- Method 540 (n=4)



7 day holding time for chlorpyrifos oxon

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M540 LCMRLs & HRLs

Analyte	LCMRL ng/L	HRL ng/L
methomyl	1.2	
3-hydroxycarbofuran	1.3	420
fenamiphos sulfone	1.0	
fenamiphos sulfoxide	0.86	
phorate sulfone	0.99	
phorate sulfoxide	2.0	
disulfoton sulfoxide	2.0	
bensulide	1.2	35,000
tebufenozide	0.81	126,000
chlorpyrifos oxon	2.0	
fenamiphos	0.64	
tebuconazole	2.0	210,000

LCMRL range: 0.64–2.0 ng/L

All analytes well below the HRLs.

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Method 543: On-line SPE-LC/MS/MS (2015) Contains 8 Analytes

Method Analytes

3-hydroxycarbofuran	fenamiphos
bensulide	fenamiphos sulfone
tebuconazole	fenamiphos sulfoxide
tebufenozide	

Analytes in red are on CCL 4

- all method analytes are pesticides or pesticide degradates (except quinoline) with the potential to contaminate drinking water sources
- concentration, elution, separation all done by automation
- 1-5 mL sample volume typical
- analysis time/sample is <20 min
- high throughput

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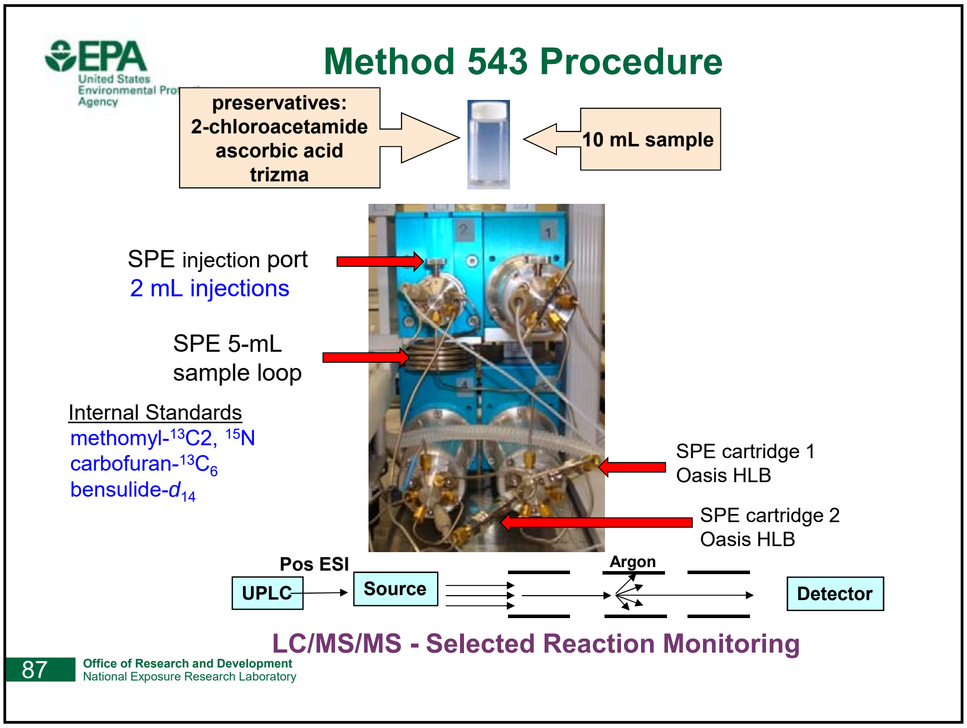
EPA United States Environmental Protection Agency
Method 543 Procedure
preservatives:
2-chloroacetamide
ascorbic acid
trizma
10 mL sample
SPE injection port
2 mL injections
SPE 5-mL
sample loop
Internal Standards
methomyl-13C2, 15N
carbofuran-13C6
bensulide-d14
SPE cartridge 1
Oasis HLB
SPE cartridge 2
Oasis HLB
Pos ESI
UPLC
Source
Argon
Detector
LC/MS/MS - Selected Reaction Monitoring
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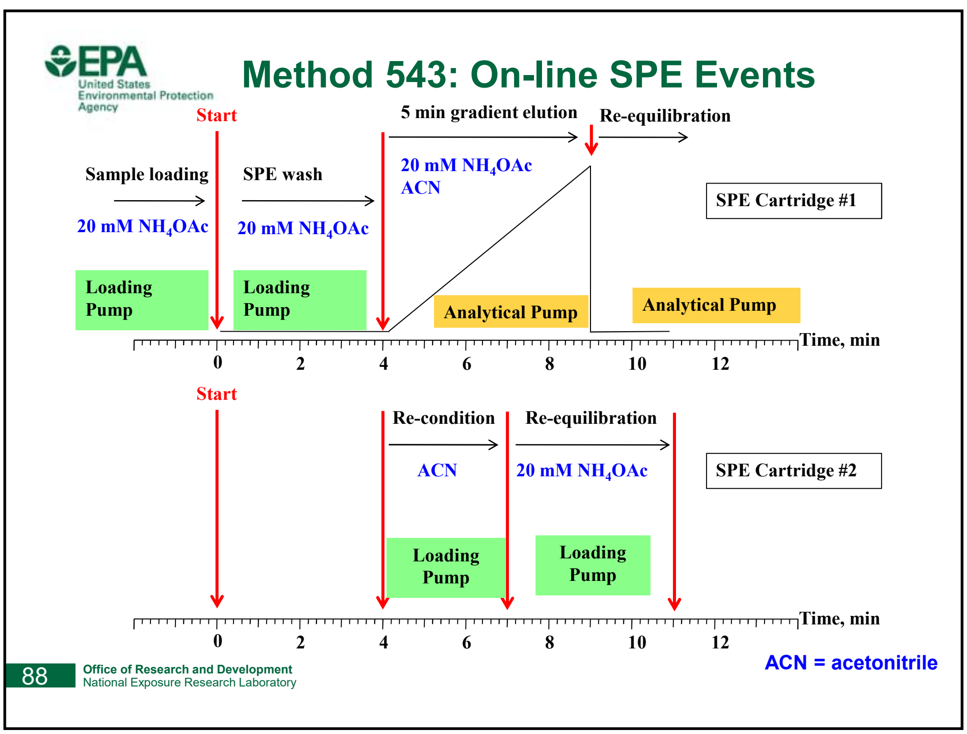
EPA United States Environmental Protection Agency
Method 543: On-line SPE Events
Start
5 min gradient elution
Re-equilibration
Sample loading
SPE wash
20 mM NH4OAc
ACN
20 mM NH4OAc
20 mM NH4OAc
SPE Cartridge #1
Loading Pump
Loading Pump
Analytical Pump
Analytical Pump
Time, min
0 2 4 6 8 10 12
Start
Re-condition
Re-equilibration
ACN
20 mM NH4OAc
SPE Cartridge #2
Loading Pump
Loading Pump
Time, min
0 2 4 6 8 10 12
ACN = acetonitrile
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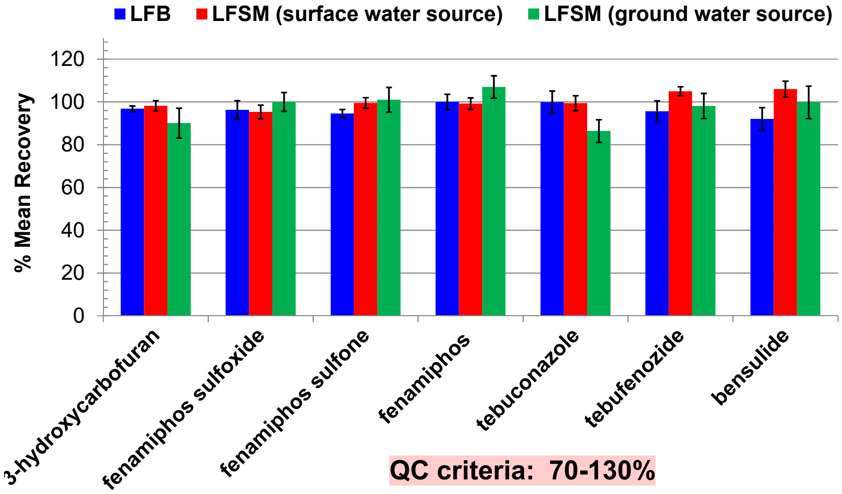
M543 Performance data

LFBs & LFSMs fortified at 20-50 ng/L (n=5)



QC criteria: 70-130%

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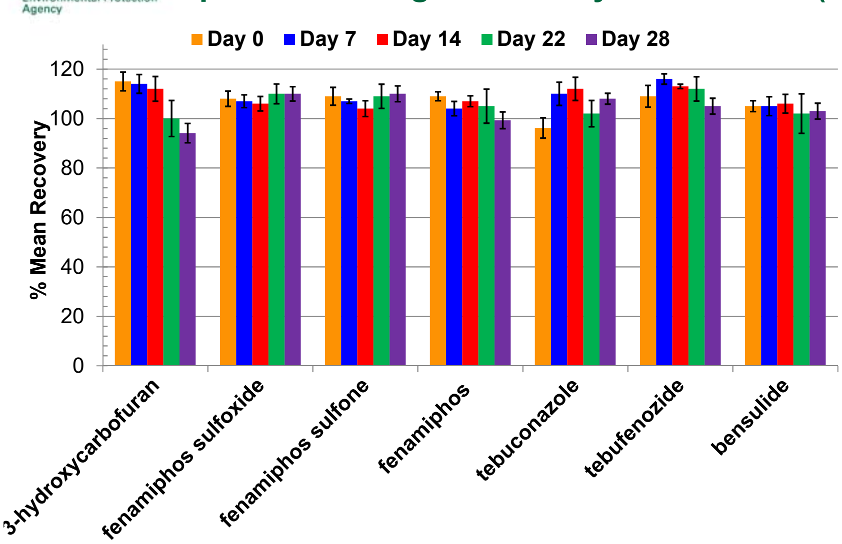
Aqueous Holding Time Study- Method 543 (n=5)



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M540 & 543 HRL and LCMRL Comparison

Analytes	HRL, ng/L	LCMRLs, ng/L Method 540	LCMRLs, ng/L On-line
3-hydroxycarbofuran	420	1.3	1.7
bensulide	35,000	1.2	1.2
tebufenozide	126,000	0.81	0.47
tebuconazole	210,000	2.0	1.3
fenamiphos		0.64	0.27
fenamiphos sulfone		1.0	1.4
fenamiphos sulfoxide		0.86	1.2

LCMRLs obtained by on-line method are below the HRLs for all analytes and similar to Method 540.

250 mL sample
0.64-2.0 ng/L

2 mL sample
0.27-1.7 ng/L

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Method 537: SPE-LC/MS/MS
14 Perfluorinated Alkyl Acids (PFAA)

Perfluorocarboxylic acids (9) Perfluorosulfonates (3)
Perfluorosulfonamidoacetic acids (2)

Method Analytes on CCL 4
PFOA – perfluorooctanoic acid
PFOS – perfluorooctane sulfonic acid

Method Analytes in UCMR 3
PFOA – perfluorooctanoic acid
PFHpA – perfluoroheptanoic acid
PFNA – perfluorononanoic acid
PFOS – perfluorooctane sulfonic acid
PFHxS – perfluorohexanesulfonic acid
PFBS – perfluorobutanesulfonic acid

Challenges: wide range of water solubilities (C_4-C_{14}), laboratory and field blank contamination, LC contamination

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Update to Method 537 (in progress)

Potential PFAS additions	Acronym	CAS#
Perfluoro-2-propoxypropanoic acid	**GenX**	13252-13-6
Nonafluoro-3,6-dioxaheptanoic acid	**NFDHA**	151772-58-6
Perfluoro (2-ethoxyethane) sulfonic acid	**PFEESA**	113507-82-7
Perfluoro-4-methoxybutanoic acid	**PFMBA**	863090-89-5
Potassium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate	**11Cl-PF3OUdS**	83329-89-9
Potassium 9-chlorohexadecafluoro-3-oxanone-1-sulfonate	**9Cl-PF3ONS**	73606-19-6
Sodium dodecafluoro-3H-4,8-dioxanonate	**ADONA**	958445-44-8

Challenge: **Obtain performance data, write method, conduct multi-lab verification and peer review method ASAP**

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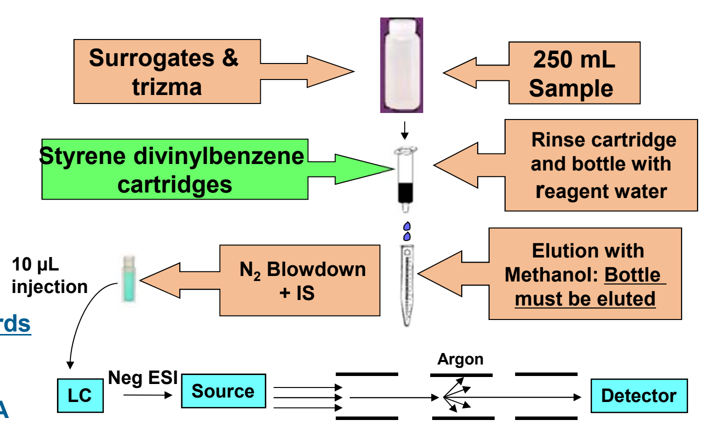
M537 Approach

Preservative
trizma

Surrogates
$^{13}C_2$-PFHxA
$^{13}C_2$-PFDA
d_5-NEtFOSAA
^{13}C-GenX

Internal Standards
$^{13}C_2$-PFOA
$^{13}C_4$-PFOS
d_3-NMeFOSAA



LC/MS/MS - Selected Reaction Monitoring

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Conclusions

- Four published methods available/expected for usage in future monitoring for unregulated contaminants
  - ✓ Pesticides
  - ✓ Pesticide degradates
  - ✓ Additional PFAS(s)
- Methods contain preservation, holding times and QC
- Performance data demonstrated
- Update to Method 537 expected to contain GenX

Questions?

Analysis of Select PFAS Compounds in Drinking Water

Steve Wendelken, Ph.D.

U.S. EPA
Office of Ground Water and Drinking Water, Technical Support Center

Initial Method Development Objectives

- Develop a SPE LC/MS/MS method for the analysis of "short chain" [perfluorinated acids, sulfonates and mono/poly perfluorinated ethers] in drinking water
  - "short chain" representing no PFAS greater than C_{12} due to physicochemical disparities
- Initially targeting method reporting levels ≤ 10 ng/L
- Extend the method to include surface water and non-potable groundwater as time and resources permit

Initial Target Analyte List

	Compound	Abbreviation	CAS	Formula (anion)	m/z (anion)
1	Perfluorobutanoic acid	PFBA	375-22-4	$C_4F_7O_2$	213
2	Perfluoro-3-methoxypropionic acid	PFMPA	377-73-1	$C_4F_7O_3$	228.974
3	Perfluoropentanoic acid	PFPeA	2706-90-3	$C_5F_9O_2$	263
4	Perfluoro-4-methoxybutanoic acid	PFMBA	863090-89-5	$C_5F_9O_3$	278.97
5	Perfluoro (2-ethoxyethane) sulfonic acid	PFEESA	113507-82-7	$C_4F_9O_4S$	315
6	Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	151772-58-6	$C_5F_9O_4$	295
7	Perfluoro-2-propoxypropanoic acid	GenX	13252-13-6	$C_6F_{11}O_3$	328.97
8	Dodecafluoro-3H-4,8-dioxanonanoate	ADONA	958445-44-8	$C_7HF_{12}O_4$	377
9	9-chlorohexadecafluoro-3-oxanonane-1-sulfonate	9Cl	73606-19-6	$C_8F_{16}SO_4Cl$	531/533
10	11-chloroeicoafluoro-3-oxaundecane-1-sulfonate	11Cl	83329-89-9	$C_{10}F_{20}SO_4Cl$	631/633

Initial Weak Anion Exchange SPE Results with Blanks

PFAS fortified "high TOC" finished drinking water

50 ng/L PFAS fortification
Sample preserved with 5 g/L Trizma
100 mL sample extraction on 200 mg UCT WAX SPE
Eluted w/ 2X5 mL 2% NH4OH in MeOH. Diluent 75% MeOH.
All values in ng/L

	Rep A	Rep B	Rep C	Matrix Blank	LRB	Average Recovery %	%RSD
PFBA	55	58	55	7	5	112	3.2
PFMPA	49	51	54	1	1	103	5.3
PFPeA	45	45	47	0	1	91	2.3
PFMBA	45	45	46	1	1	90	1.3
EESA	49	45	47	0	0	94	4.1
NFDHA	44	46	47	0	0	91	2.3
GenX	43	43	44	0	0	87	2.1
ADONA	45	47	46	0	0	92	1.6
9Cl	50	50	51	0	0	101	1.6
11Cl	49	50	51	0	0	100	1.7

Expanding PFAS Target List

- Currently evaluating expanding the target list to create a more "universal" PFAS method that includes most Method 537 targets along with other analytically feasible PFAS.
- New method focused on highest analytical performance for priority short chain perfluorinated acids, sulfonates and mono/poly perfluorinated ethers.
- Any additional PFAS targets must have an available analytical standard.
- Final target list may include closer to two dozen or more PFAS.

General Method Procedure

- Samples collected in polypropylene bottles
- Samples preserved with Trizma
- SPE extraction with weak anion exchange media
- SPE eluted with basic
- Extracts analyzed by LC/MS/MS
- Target MRLs ≤ 10 ng/L

Questions

EPA Method in Development 558

Alan Zaffiro Ph.D.

APTIM
Contractor to U.S. EPA

Method 558 (GC/MS)

N-Methyl-2-pyrrolidone (In Development)
Ethyl carbamate/Urethane (In Development)

Purple: CCL 4

Initial Contaminants Considered (Method 558)

- Water-soluble, low-molecular weight, and priority contaminants remaining on CCL 4 for analysis via GC/MS
  - acetamide, aniline, ethyl carbamate (urethane), ethylene glycol, ethylene oxide, hydrazine, methanol, N-methyl-2-pyrrolidone, propylene oxide, triethylamine, N-nitrosodiphenylamine and 4,4'-methylenedianiline
- Investigated via literature search and laboratory experiments
  - contaminant properties
  - potential extraction techniques
  - chromatographic efficiency
  - programmed temperature and split/splitless injection

Selection of Target Analytes for Method 558

- Proposed multi-analyte method for:
  - 4,4'methylenedianiline, urethane, N-methyl-2-pyrrolidone, aniline, p-chloroaniline, 2,4-dichloroaniline, 2,4,6-trichloroaniline, and N-nitrosodiphenylamine
- Requires tandem Solid Phase Extraction (SPE) and results in 3 separate extracts for GC/MS
- Settled on two priority contaminants
  - Ethyl carbamate and N-methyl-2-pyrrolidone
  - Common SPE cartridge and GC column

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Draft Method 558

- Analytes
  - ethyl carbamate (HRL = 0.035 µg/L) and N-methyl-2-pyrrolidone (HRL = 4,200 µg/L)
  - ABILITY TO MEET MONITORING GOALS: MRLs confirmed at 0.0175 µg/L in our laboratory for both contaminants without extract concentration
  - Attempted 4:1 extract concentration as option to achieve lower MRL, but abandoned because of inconsistent performance
- Surrogate compounds
  - Ethyl-d_5 carbamate and N-methyl-2-pyrrolidone-d_9
- Internal standard
  - 1,4-dichlrobenzene-d_4

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Method 558 Parameters

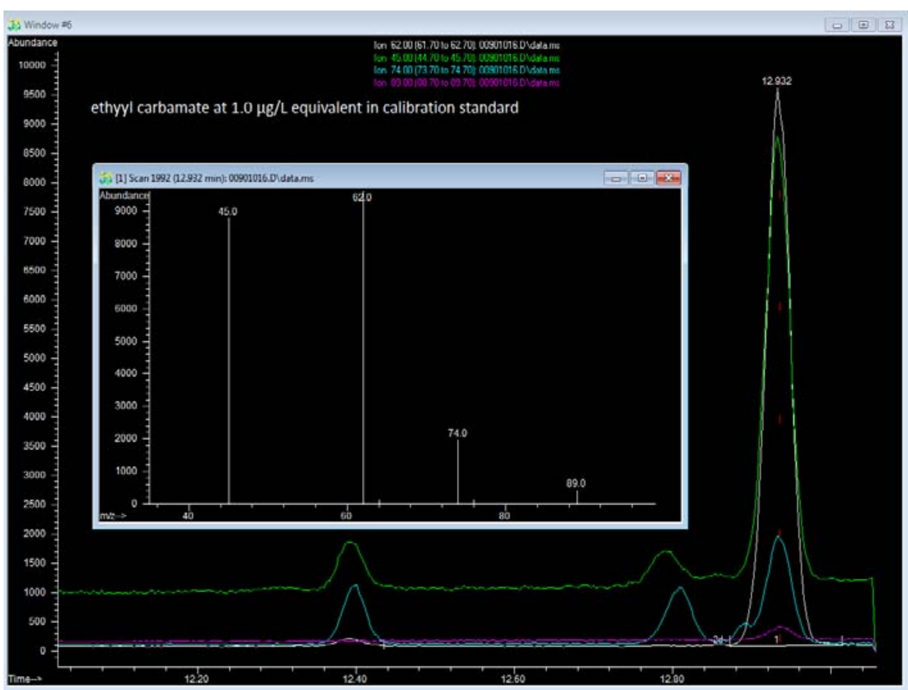
- **Preservation:** identical to EPA Methods 522 and 541
  - 1 g/L $NaHSO_4$ and 50 mg/L Na_2SO_3
- **SPE:** 0.5 L Sx; neutralized with 25 mL x 0.8 M $NaHCO_3$; Waters (Milford, MA) AC-2 (400 mg); 150 µL MeOH rinse followed by 5-min N_2 dry @ 5 L/min; elution with 150 µL MeOH followed by 2.3 mL ethyl acetate; 2-mL final extract volume
- **Extract analysis:** 30 m x 0.25 mm i.d. x 0.5 µm d_f WAX column; 1 µL pulsed-pressure injection @ 200 °C inlet; temperature-programmed separation; MS detection in SIM mode

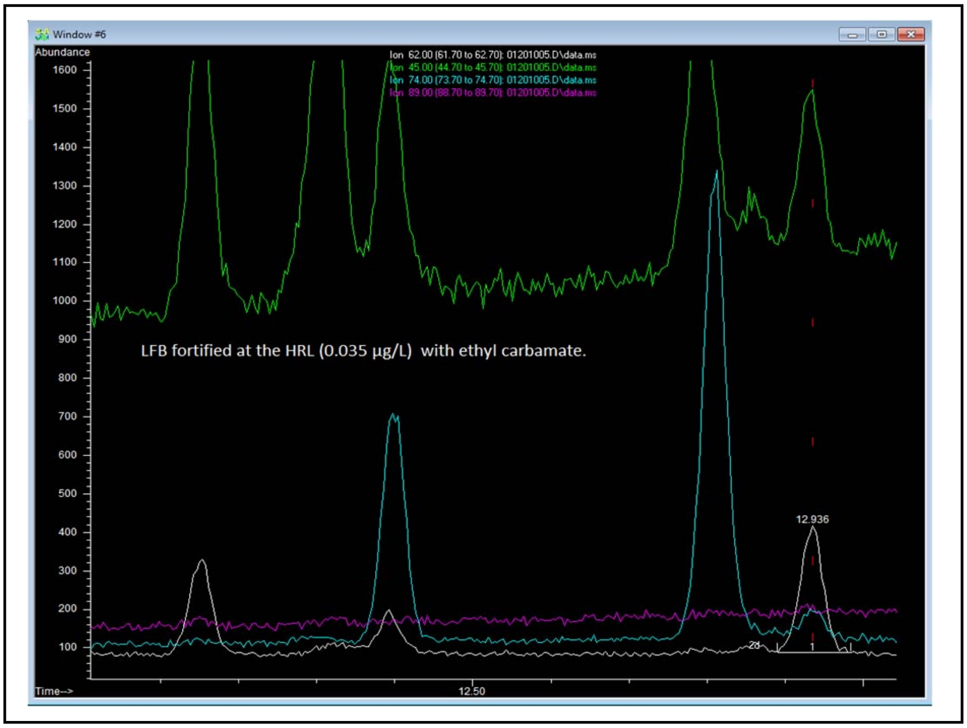
Measurement Ranges

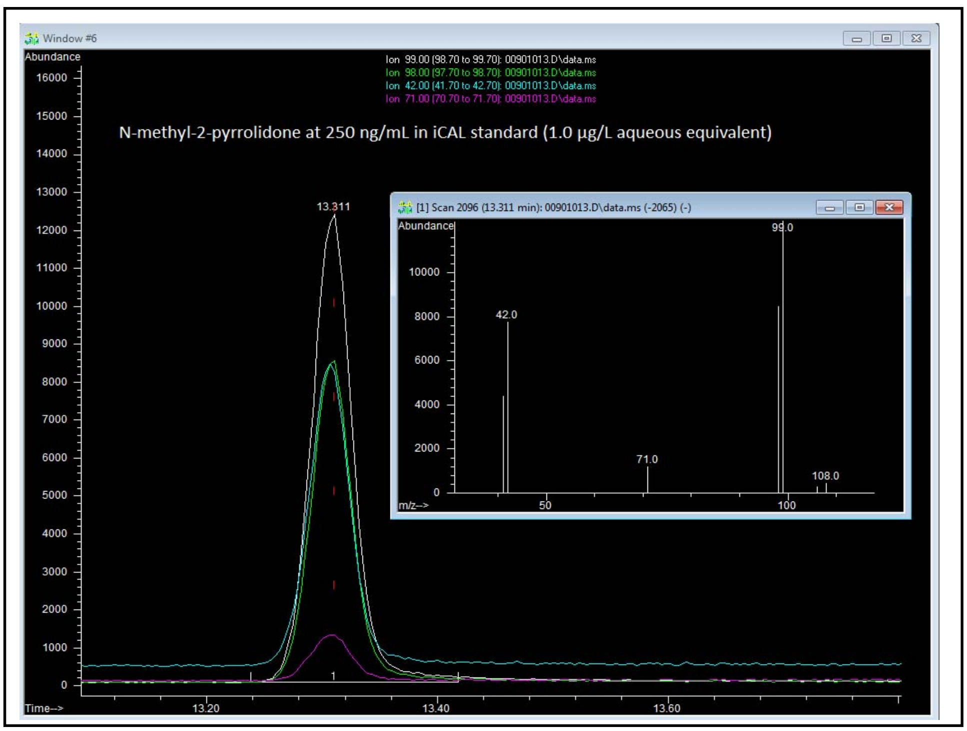
Analyte / QC Compound	Range without extract concentration, µg/L
Ethyl carbamate	0.0175–2.0
N-methyl-2-pyrrolidone	0.0175–2.0
Ethyl-d_5 carbamate, surrogate	0.25
N-methyl-2-pyrrolidone-d_9, surrogate	0.25

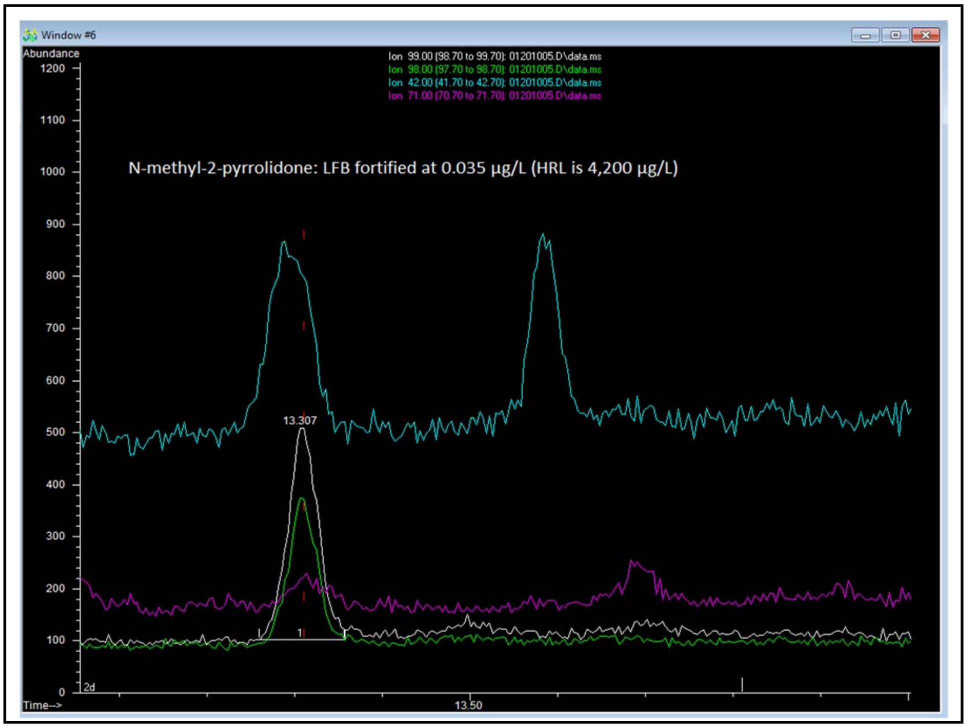
Future Work Method 558

- Evaluate other activated carbon cartridges
  - Must be reversible cartridge
  - UCT (Bristol, PA) 500 mg cartridge in progress
- Collect method performance data for all extraction formats
  - Waters AC-2 in progress
- Outside laboratory validation
  - At least two laboratories, preferably three or more
- The following ion chromatograms illustrate the chromatographic peak profiles, the ions monitored, and demonstrate that at least one confirmation ion is detected at 0.035 µg/L




Window #6
Abundance
Ion 62.00 (61.70 to 62.70): 01201005.D\data.ms
Ion 45.00 (44.70 to 45.70): 01201005.D\data.ms
Ion 74.00 (73.70 to 74.70): 01201005.D\data.ms
Ion 89.00 (88.70 to 89.70): 01201005.D\data.ms
LFB fortified at the HRL (0.035 µg/L) with ethyl carbamate.
12.936
Time-->
12.50


Window #6
Abundance
Ion 99.00 (98.70 to 99.70): 00901013.D\data.ms
Ion 98.00 (97.70 to 98.70): 00901013.D\data.ms
Ion 42.00 (41.70 to 42.70): 00901013.D\data.ms
Ion 71.00 (70.70 to 71.70): 00901013.D\data.ms
N-methyl-2-pyrrolidone at 250 ng/mL in iCAL standard (1.0 µg/L aqueous equivalent)
13.311
[1] Scan 2096 (13.311 min): 00901013.D\data.ms (-2065) (-)
99.0
42.0
71.0
108.0
m/z-->
Time-->
13.20
13.40
13.60


Window #6
Abundance
Ion 99.00 (98.70 to 99.70): 01201005.D\data.ms
Ion 98.00 (97.70 to 98.70): 01201005.D\data.ms
Ion 42.00 (41.70 to 42.70): 01201005.D\data.ms
Ion 71.00 (70.70 to 71.70): 01201005.D\data.ms
N-methyl-2-pyrrolidone: LFB fortified at 0.035 µg/L (HRL is 4,200 µg/L)
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
13.307
Time-->
13.50

Questions

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DW Stakeholder
June 6, 2018

Development of U. S. EPA Method 559 for the Analysis of Nonylphenol in Drinking Water by Solid Phase Extraction and LC/MS/MS

Daniel R. Tettenhorst and Jody A. Shoemaker

Office of Research and Development
National Exposure Research Laboratory

EPA United States Environmental Protection Agency

Method Development Goals

- **70-130% recovery with <30% RSD**
- **Laboratory Reagent Blank (LRB) no more than 1/3 the Minimum Reporting Level (MRL)**
- **Preservation**
  - ✓ **Dechlorinating Agent**
  - ✓ **Antimicrobial**
- **Establish sample and extract holding times – ideally ≥14 days**
- **Lowest concentration minimum reporting limits (LCMRLs) goal – less than health reference level (HRL)**
- **HRL for Nonylphenol is 105 µg/L**

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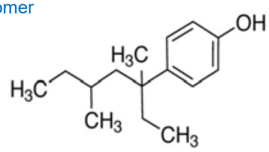
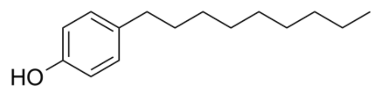
What is Nonylphenol?

- Nonylphenol (NP) is used to make plastics, detergents, paints, pesticides, personal care products
- Many products have “down the drain” applications and are flushed into the water supply

Technical, Branched Nonylphenol	Linear Nonylphenol
Mostly branched C9-alkyl phenols	Linear C9 chain
CAS# 84852-15-3	CAS# 104-40-5
Best represents commercially produced NP found in environment	Laboratory generated chemical not found in environment

One example of a 4-branched-NP isomer





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Method will report technical, branched NP, CAS #84852-15-3

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Drinking Water Procedure

Amber Glass Bottle

250 mL sample

Preservatives
Sodium bisulfate = antimicrobial
Ascorbic acid = dechlor

Possible Surrogates
4-n-Nonylphenol-$^{13}C_6$ (linear)
4-n-Nonylphenol-2,3,4,5-d_4, OD (linear)
4-n-Octyl-d_{17}-phenol (linear)
4-tert-Octylphenol-$^{13}C_6$ (branched)

Oasis HLB
Bond Elut C18
Strata-X
100-200 mg

Rinse cartridge with reagent water and dry under vacuum

Rinse and elute sample bottle with 2 X 2 mL acetone

Add IS

Adjust to 5 mL final volume with acetone

10 µL injection

LC/MS/MS Neg ESI

Internal Standard
4-(1,3-Dimethyl-1-ethylpentyl) phenol-$^{13}C_6$

Optimized Conditions

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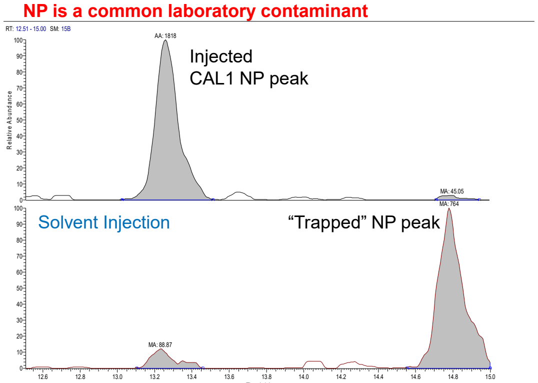
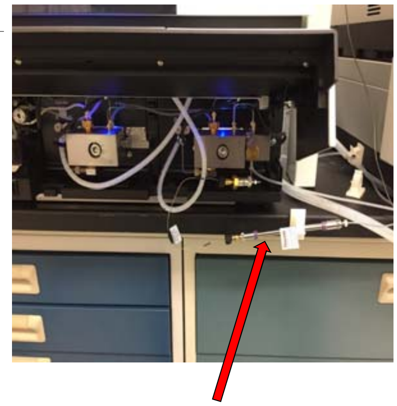
System Background Contribution

NP is a common laboratory contaminant





Trapping column used to separate LC system contamination away from injected NP peak

Trapping Column

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Mobile Phase Conditions

- **Sensitivity increases as mobile phase pH approaches the pKa (10.28)**
- **Ammonium hydroxide (0.01%) used to increase pH to 10**
  - **Still 1 - 2 pH units below upper pH limit of C18 LC columns**
- **Additional mobile phase modifiers resulted in loss of NP sensitivity**

Standard Concentration	NP Area	Summary of Conditions
150 µg/L	2573	0.01% acetic acid in A/B
150 µg/L	3621	5 mM ammonium acetate in A/B pH = estimate 6.5
150 µg/L	18437	no modifiers/neutral pH
150 µg/L	323825	0.01% ammonium hydroxide in A/B pH = 10.05
150 ug/L	???	0.1-0.5 mM ammonium fluoride in A

Mobile Phase	A	B
Time (min)	DI water No modifiers	Methanol No modifiers
Initial	80	20
15	5	95
19	5	95
19.1	80	20
23	80	20

Thermo Hypersil Gold C18, 2.1 x 50 mm, 3 µm, 0.3 mL/min flowrate, 10 µL injection

Electrospray Conditions	
Polarity	Negative Ion
Capillary needle voltage	-4.0 kV
Sheath Gas	40 L/h
Aux Gas	4 L/h
Sweep Gas	2 L/h
Ion Transfer Tube Temp	325 °C
Vaporizer Temp	375 °C

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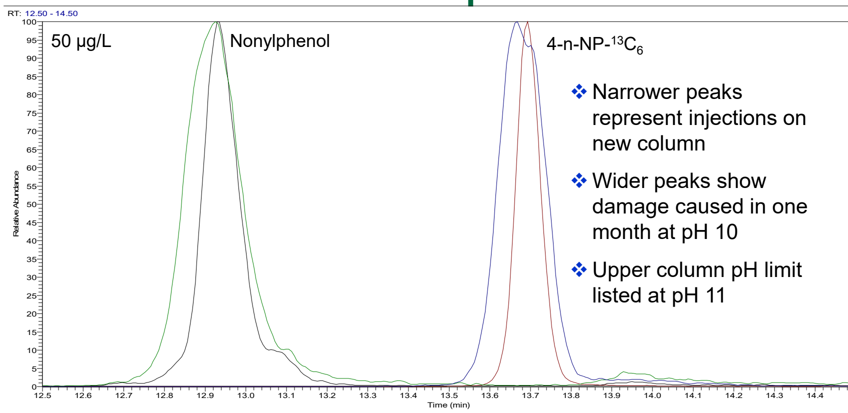
Degradation of C18 Analytical Column at pH 10



- Narrower peaks represent injections on new column
- Wider peaks show damage caused in one month at pH 10
- Upper column pH limit listed at pH 11

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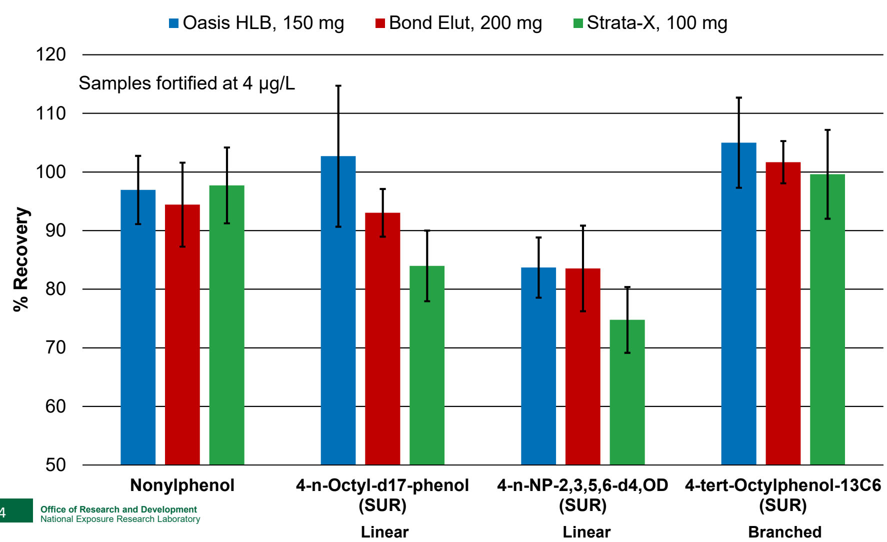
Mean Recovery and Precision (n=4) of Laboratory Fortified Blanks with Various SPE Sorbents



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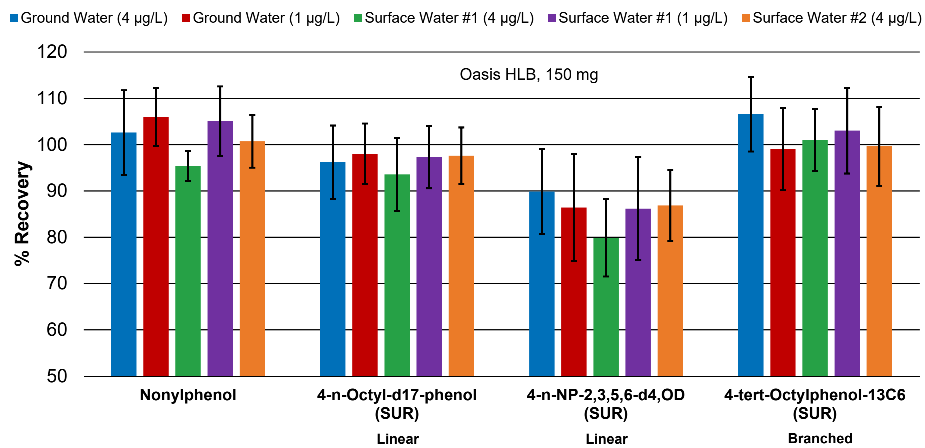
Mean Recovery and Precision (n=4) in Laboratory Fortified Drinking Water Samples



- ❖ Results meet DQOs of 70-130%

Surface Water #1 TOC = 2.2 mg/L, Hardness = 103 mg/L

Surface Water #2 TOC = 0.97 mg/L, Hardness = 120 mg/L

Ground Water TOC = 0.55 mg/L, Hardness = 360 mg/L

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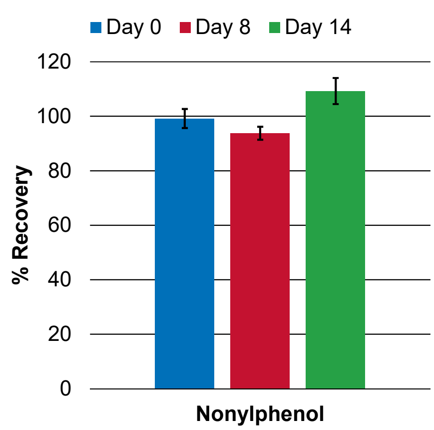
Preliminary Aqueous Holding Time Study Mean Recovery and Precision (n=4)

Tap Water Fortified at 4 µg/L, Oasis HLB, 150 mg



- ❖ Sodium bisulfate and ascorbic acid preservation
- ❖ Preliminary study to 14 days
- ❖ A longer study to 28 days will be performed
- ❖ Extract holding time will be studied as well

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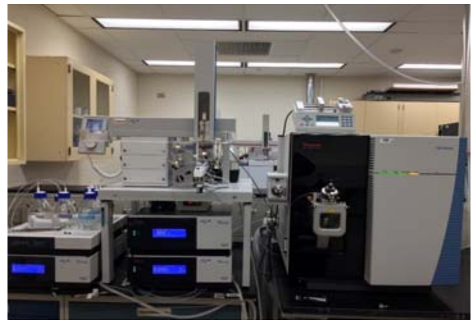
Detection Limit

Analyte	µg/L
	DL
Nonylphenol	0.08



Reference Guidelines

Guideline	Limit
HRL	105 µg/L
Minnesota Department of Health	20 µg/L

- ❖ **Preliminary Detection Limit (DL) based on precision only**
- ❖ **LCMRL to be developed based on precision and accuracy**

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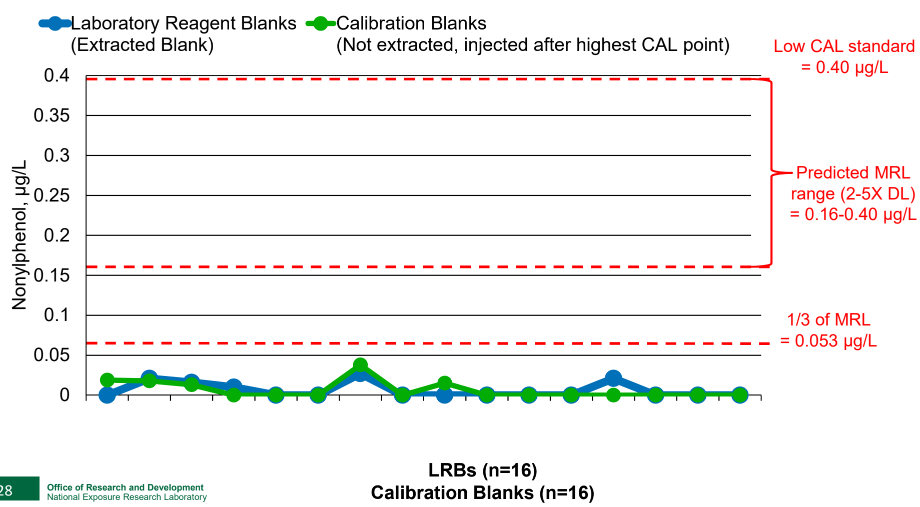
Predicting Blank Contamination Problems



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Summary

- Rugged, standardized, sensitive method developed for nonylphenol in drinking water
- Investigated best labeled SUR and IS standards for method, chose branched octylphenol for SUR and branched nonylphenol for IS
- Meets data quality objectives (DQOs) for several types of SPE cartridges, for a ground water source and surface water sources, and for blank recovery
- On target to easily meet HRL

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Future Work and Method Delivery

- Investigate ammonium fluoride as LC mobile phase modifier to increase sensitivity and evaluate its impact on ESI stability in different DW matrices
- Include octylphenol as an additional analyte provided DQOs can be met
- Final performance data including holding time study and LCMRL study to be performed spring-summer of 2018
- Final peer reviewed, multi-laboratory validated method published by September, 2019

United States Environmental Protection Agency

Questions??

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Lunch

(1 hour)

United States Environmental Protection Agency

Method in Development: Legionella

Maura J. Donohue Ph.D.

Legionellaceae

- Legionella (Genus)
  - Gram negative bacteria (Gammaproteobacteria)
  - Flagella rod (2-20 µm)
  - Slow grower (3 to 10 days)
  - Majority of species will grow in free-living amoebae
  - Aerobic, L-cysteine and iron salts are required for in vitro growth, pH: 6.8 to 7, Temp: 25 to 43 ° C
  - ~65 species
  - Pathogenic or potentially pathogenic for human

From Environmental Microorganism to Public Enemy

Genus	65 Species	Disease

Legionella spp.

¤ Legionella, genus.	Legionella maceachernii
Legionella adelaidensis	Legionella massiliensis
Legionella anisa	Legionella micdadei
Legionella beliardensis	Legionella moravica
Legionella birminghamensis	Legionella nagasakiensis
Legionella bozemanae corrig.	Legionella nautarum
Legionella brunensis	Legionella norrlandica
Legionella busanensis	Legionella oakridgensis
Legionella cardiaca	Legionella parisiensis
Legionella cherrii	Legionella pittsburghensis.
Legionella cincinnatiensis.	Legionella pneumophila
Legionella drancourtii	Legionella quateirensis
Legionella dresdenensis	Legionella quinlivanii
Legionella drozanskii	Legionella rowbothamii
Legionella dumoffii.	Legionella rubrilucens
Legionella erythra	Legionella sainthelensi
Legionella fairfieldensis	Legionella santicrucis
Legionella fallonii	Legionella saoudiensis
Legionella feeleii	Legionella shakespearei
Legionella geestiana	Legionella spiritensis
Legionella gormanii	Legionella steelei
Legionella gratiana	Legionella steigerwaltii
Legionella gresilensis	Legionella taurinensis
Legionella hackeliae	Legionella thermalis
Legionella impletisoli	Legionella tucsonensis
Legionella israelensis	Legionella tunisiensis.
Legionella jamestowniensis	Legionella wadsworthii
Legionella jordanis	Legionella waltersii
Legionella lansingensis	Legionella worsleiensis
Legionella londiniensis	Legionella yabuuchiae
Legionella longbeachae	
Legionella lytica	

Legionella pneumophila
Brenner et al. 1979

- 2000-2014 All OUTBREAKS are associated with *Legionella pneumophila* Sg1 (Garrison et al. 2016)
- 94% of all legionellosis cases are associated with *Legionella pneumophila* Sg1

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Legionellosis: Respiratory Disease

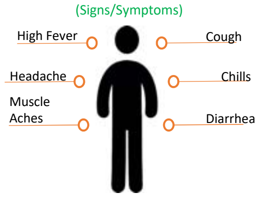
Disease

Legionellosis = pneumonia

- Legionaries' Disease (severe)
- Pontiac Fever (mild/lite)

Signs/Symptoms

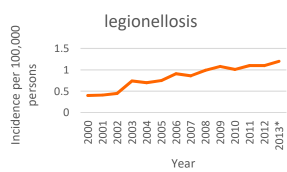
Pneumonia
(Signs/Symptoms)



National Notifiable Disease Surveillance System (NNDSS)

2012 National Reportable Disease List: contained the names of 110 Diseases/Microorganisms

Number of Cases Reported in 2015:
5,100 cases



LEGIONELLOSIS.
Incidence,* by year — United States, 2000–2013, Source NNDSS

In 2012: legionellosis was ranked 23rd/110 in having the most reported cases.

Annual Cost of Treatment in the U.S.

Number of Hospitalization/year
8,000-18,000 cases
avg(13,000)

Marston, (1997)

Total Hospitalization Cost:
$433,758,000

Collier, S.A. et al 2012:
Direct healthcare costs of selected disease primarily or partially transmitted by water. Epidemiology Infection, **140**, p2003-2013

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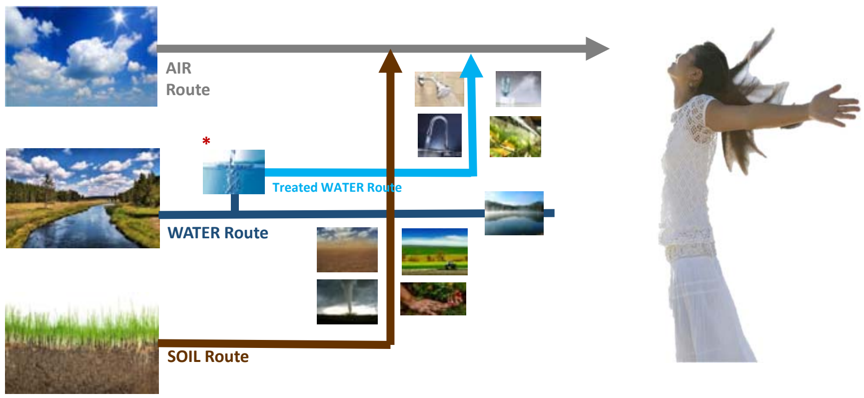
Exposure Routes: Environmental Sources

Legionella pneumophila is a microorganism of the natural environment found in soil and water.



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Inside the Agency: *Legionella*

- Environmental microorganism
- Due to treatment the likelihood of *Legionella* presence in public supply water is low.
- Gram negative bacteria are inherently prone to chemical disinfection.
- Premise plumbing issue not a public supply issue.
  - (cooling towers, decorate fountain, HVAC systems)
- Potential occurrence, maybe persistence, and never colonization.
- Distribution not premise plumbing (amount of water moved)

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Inside the Agency: *Legionella*

Surface Water Treatment Rule - 1989

SWTRs is to reduce illnesses caused by pathogens in drinking water. The disease-causing pathogens include *Legionella*, *Giardia lamblia,* and *Cryptosporidium*.

- Applies to all public water systems using surface water sources or ground water sources under the direct influence of surface water.
- Requires most water systems to filter and disinfect water Establishes maximum contaminant level goals (MCLGs) for viruses, bacteria and *Giardia lamblia.*
- Includes treatment technique (TT) requirements for filtered and unfiltered systems to protect against adverse health effects of exposure to pathogens.
- *Legionella* MCLG is 0 cfu
- Prescribes NO method nor monitoring requirements
- TT requirements (filtering and disinfection)

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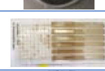
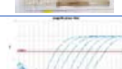
Legionella Methods

Culture- Gold Standard Standard Methods CDC ISO	BYCE CVCC GVCC		Colony Form Units	Legionella spp.
Legioleart Idexx	Yes, Proprietary		Most Probable Number (MPN)	Legionella pneumophila
DNA			Cell Equivalence Target Number	Legionella Legionella pneumophila Legionella pneumophila Sg1
RNA Sigma Aldrich: HybriScan				Legionella spp. Legionella pneumophila
Antigen Alere			Presence/Absence	Legionella pneumophila Sg1

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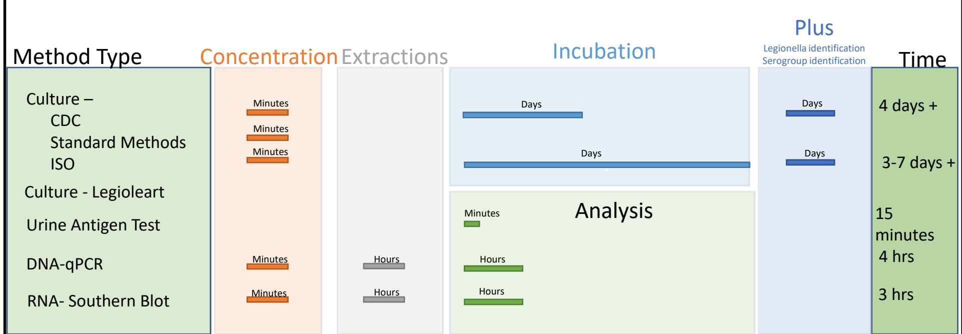
Legionella Methods- Time is of the Essence?



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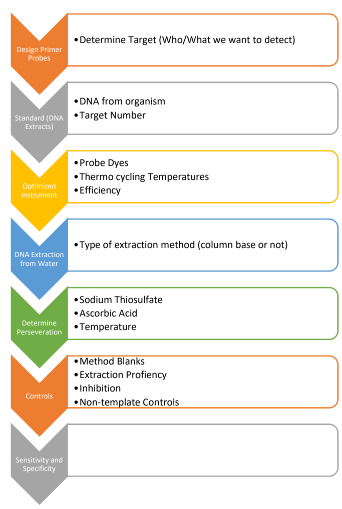
Method Verification Process

Parshionikar S, et al. 2009
Method Validation of US EPA Microbiological Methods of Analysis

Bustin SA, et al. 2009
The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. Clinical Chemistry 55 611-622p.



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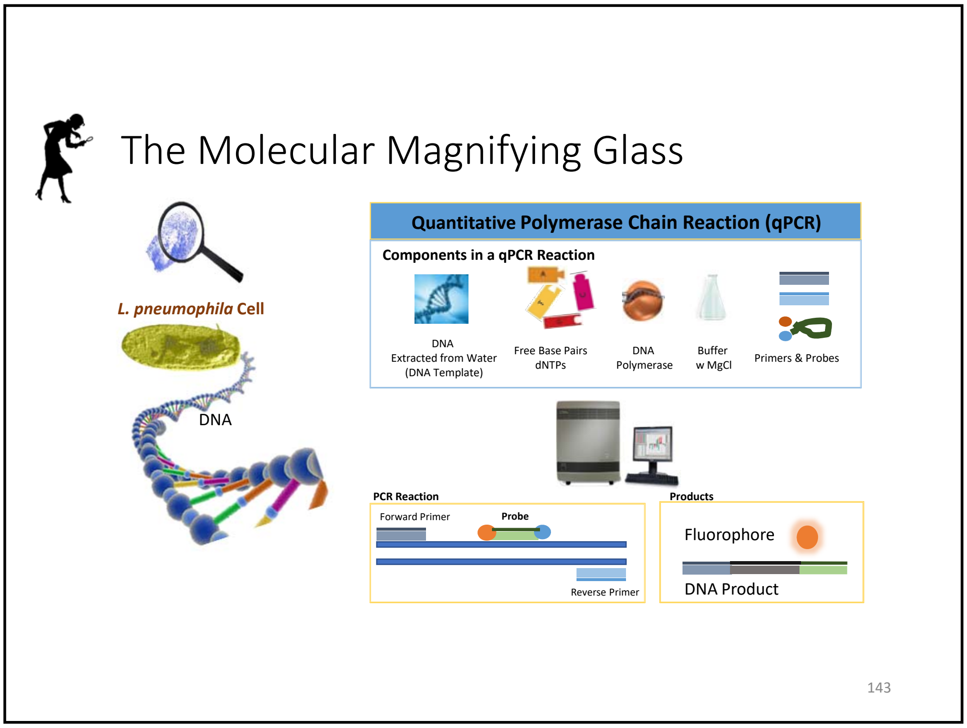
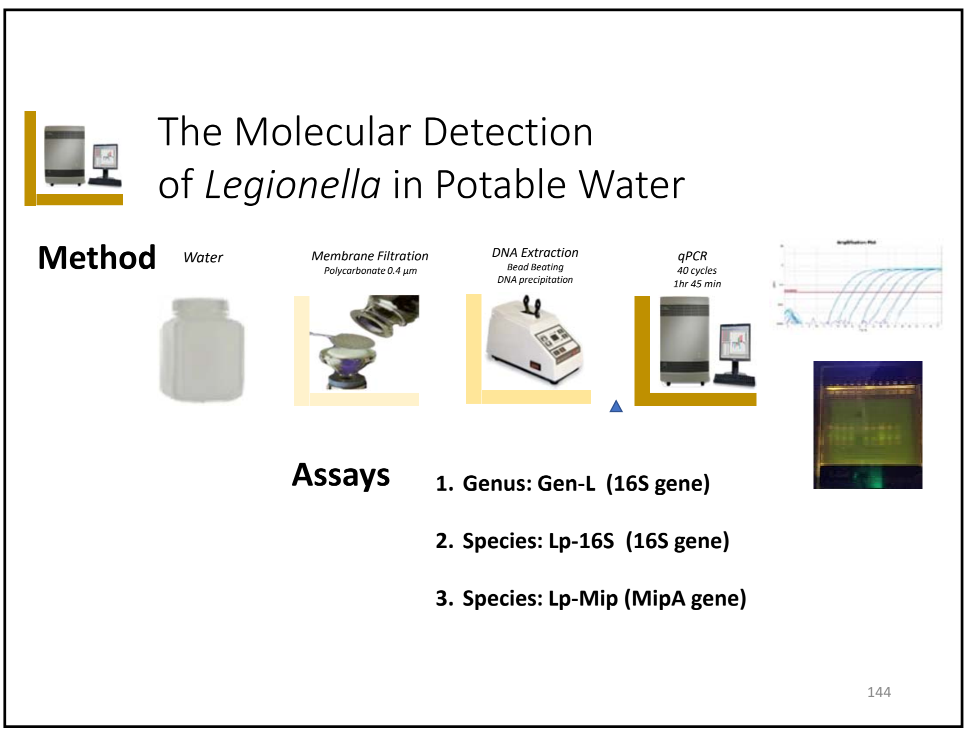

The Molecular Magnifying Glass
L. pneumophila Cell
DNA
Quantitative Polymerase Chain Reaction (qPCR)
Components in a qPCR Reaction
DNA
Extracted from Water
(DNA Template)
Free Base Pairs
dNTPs
DNA
Polymerase
Buffer
w MgCl
Primers & Probes
PCR Reaction
Forward Primer
Probe
Reverse Primer
Products
Fluorophore
DNA Product
143


The Molecular Detection
of Legionella in Potable Water
Method
Water
Membrane Filtration
Polycarbonate 0.4 µm
DNA Extraction
Bead Beating
DNA precipitation
qPCR
40 cycles
1hr 45 min
Assays
1. Genus: Gen-L (16S gene)
2. Species: Lp-16S (16S gene)
3. Species: Lp-Mip (MipA gene)
144

QC Samples for qPCR

1. **Method Blank** (negative control)
   - Sterile molecular biology grade water filtered and processed at the same time in the same way as unknowns
2. **Standards** (positive control)
   - Purified genomic DNA from target, serially diluted
3. **No Template Control** (negative control)
   - Sterile molecular biology grade water added to qPCR reaction instead of DNA extract
4. **Internal positive Control** (IC)
   - Commercially available kit (TaqMan Exogenous Internal Positive Control Kit, Life Technologies, Carlsbad, CA)

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Method in Development: Legionella (qPCR)

Phase I: Standard Curve Generation

Phase II: Extraction Proficiency

Phase III: Sensitivity Study

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Phase I:Standard Curve Generation

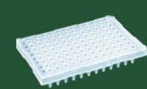
Purpose: To establish assay performance measures and analyst competence

What:

- Standard Curve (7 point of DNA dilution series).
- Each analyst will make four independent "Assay/Mastermix mixes".
- Analyst will test each Assay/Mastermix mix by analyzing each standard in triplicate.
- Include a Non Template Control (NTC).
- In total 96 Reactions were analyzed by each analyst.

Who:

- Three Internal Labs
- One External Lab



	Verification by:			
	Lab 1 (Internal)	Lab 2 (Internal)	Lab 3 (Internal)	Lab 4 (External)
Assay Designation				
Gen L	Lab 1	Lab 2		Lab 4
Lp Mip	Lab 1	Lab 2		Lab 4
Lp 16S		Lab 2	Lab 3	Lab 4

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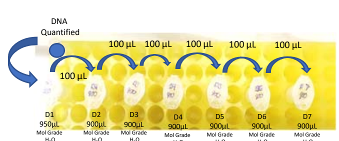
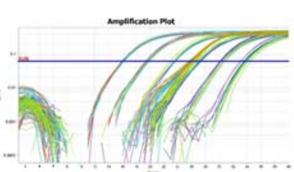
Phase I: Standard Curve Generation





Measures

- Precision
- Accuracy
- Linearity
- Relative Standard Deviation
- Efficiency
- Limit of Detection
- Limit of Quantification

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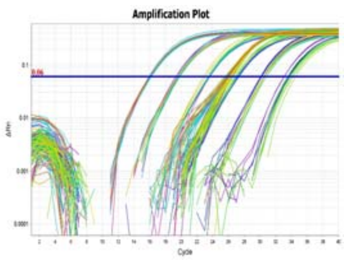
Units of Measurement: qPCR

Cq (quantitation cycle) = florescence light of the probe	Cell Equivalence	Cell (Log) Equivalence



Higher Cq= Lower the Concentration

Lower Cq= Higher the Concentration

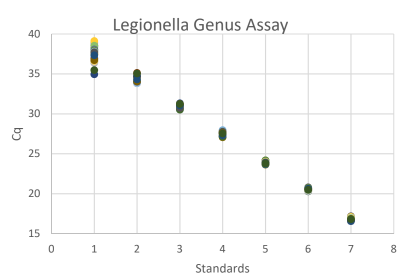
A mathematical formula generated from the standard curve (Regression Line).

- DNA extract quantified
- Mass of the genome

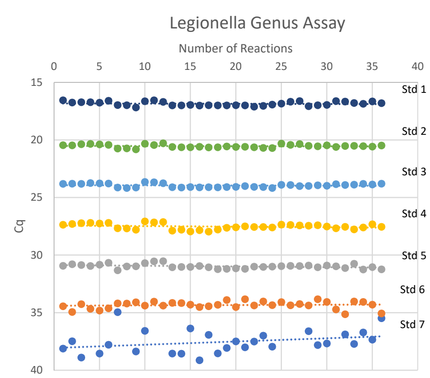
Cell Equivalence (log) transformed

149

Standard Curve: *Legionella spp.* Genus (16S)

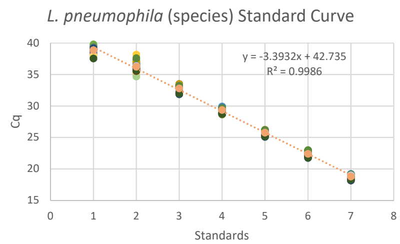


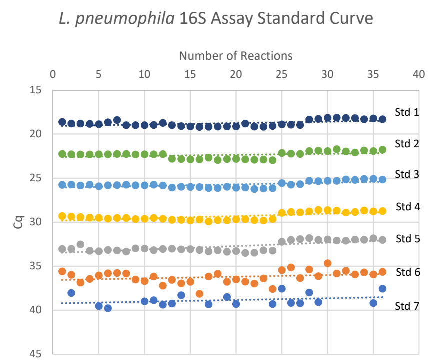
	1	10^1	10^2	10^3	10^4	10^5	10^6
mean	37.55	34.35	30.98	27.53	23.96	20.54	16.86
STDev	0.98	0.32	0.18	0.24	0.15	0.13	0.18
% Det	77%	100%	100%	100%	100%	100%	100%
Cv	0.03	0.01	0.01	0.01	0.01	0.01	0.01
%RSD	2.62	0.93	0.60	**0.87**	0.65	**0.64**	1.04



150

Standard Curve: *L. pneumophila* (species) (16S)





	1	10^1	10^2	10^3	10^4	10^5	10^6
mean	38.87	36.23	32.78	29.36	25.74	22.38	18.78
STDev	0.72	0.70	0.60	0.45	0.36	0.40	0.37
% Det	53%	100%	100%	100%	100%	100%	100%
Cv	0.02	0.02	0.02	0.02	0.01	0.02	0.02
%RSD	1.84	1.94	1.84	**1.52**	1.41	**1.80**	1.98

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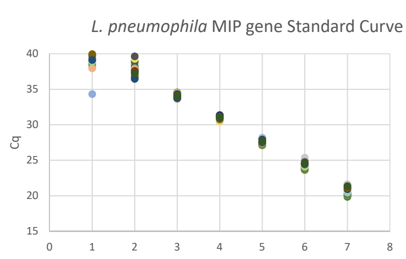
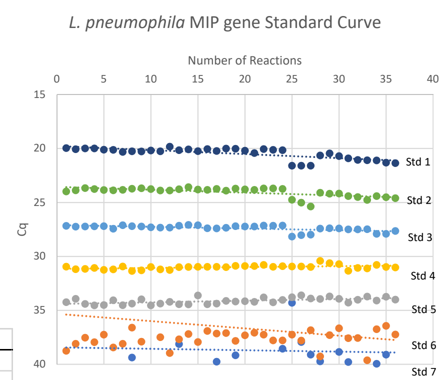
Standard Curve: *L. pneumophila* (species) (MIPA)





	1	10^1	10^2	10^3	10^4	10^5	10^6
mean	38.75	37.62	34.13	31.00	27.39	24.05	20.46
STDev	1.48	0.73	0.28	0.19	0.28	0.43	0.53
% Det	36%	100%	100%	100%	100%	100%	100%
Cv	0.04	0.02	0.01	0.01	0.01	0.02	0.03
%RSD	3.81	1.95	0.82	**0.63**	1.04	**1.81**	2.60

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Phase I: qPCR Method Performance Criteria

			Leg-G	Lp-16S	Lp-MipA
• Precision (RSD) repeatability					
	Analyst II		0.9-3.5	0.1-1.5	0.6-3.8
	Analyst IV	0.3-2.1	0.4-2.1	0.6-4.8	
• Precision (RSD) reproducibility		0.6-2.6		1.0-2.9	
• Linearity (R^2)			0.9997	0.9986	0.9897
• Efficiency (E)			94.7	97.1	106
• Limit of Detection (LOD)			10 ce/rx	10 ce/rx	100 ce/rx
• Limit of Quantification (LOQ)		100 ce/rx	100 ce/rx	100 ce/rx	

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Phase II: Extraction Proficiency

Purpose: to define Extraction Proficiency

Spiked filters with known quantities are given to each Lab for extraction and analysis

What:

- 3 Concentrations + Method Blank
- 3 spike filters/concentration (Total: 9 Extractions)
- Each Extraction analyzed in triplicate.

Who:

- Three Internal Labs
- One External Lab

Theoretical Amount (NO Membrane and Extraction) ⟷ Extracted Amount (Membrane and Extraction)

Percent (%) Mean Recovery

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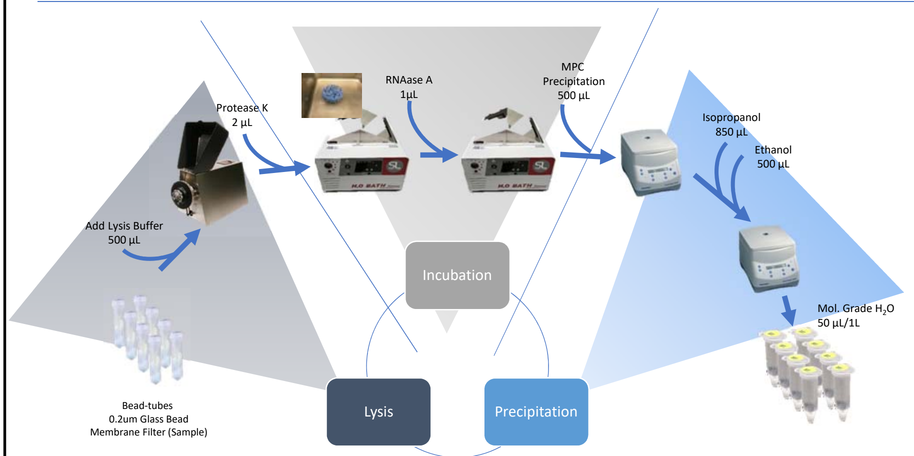
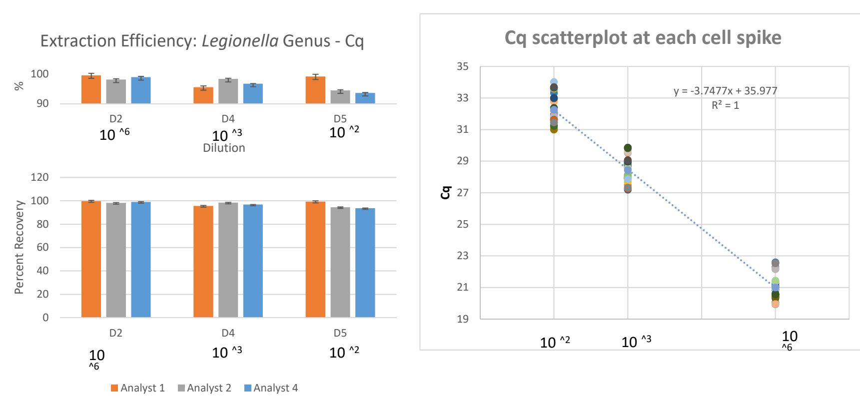
Sample Extraction



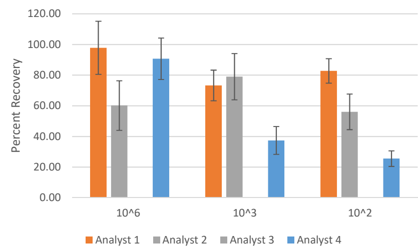
155

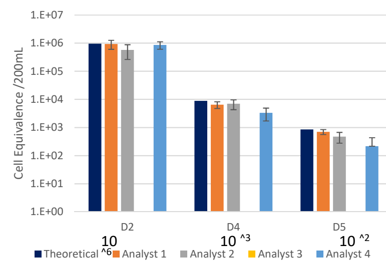
Extraction Efficiency: *Legionella spp.* Genus



156

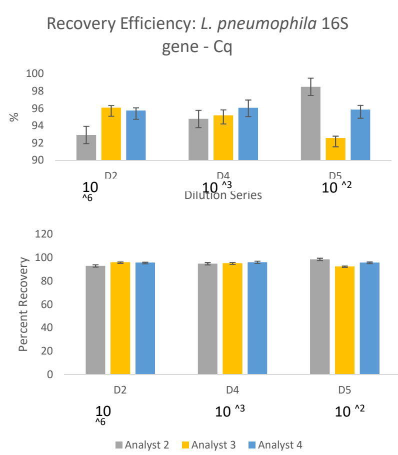
Extraction Efficiency: *Legionella spp.* Genus – Cell Equivalence/200mL

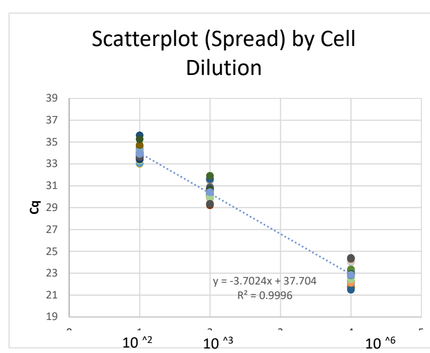




157

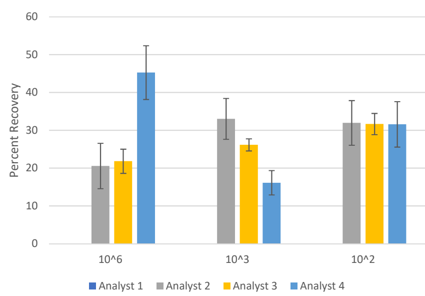
Extraction Efficiency: *L. pneumophila*– Cq/200 mL

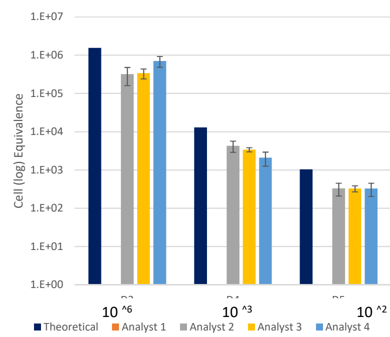




158

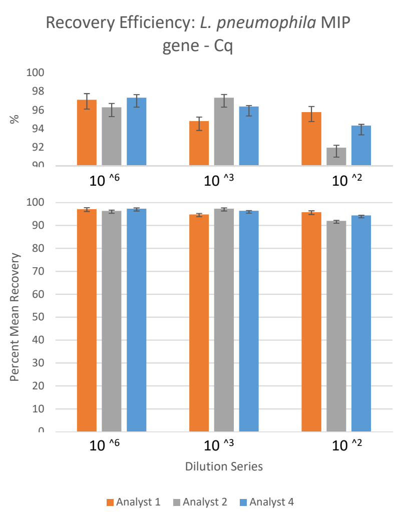
Extraction Efficiency: *L. pneumophila*– Cell Equivalence/200mL

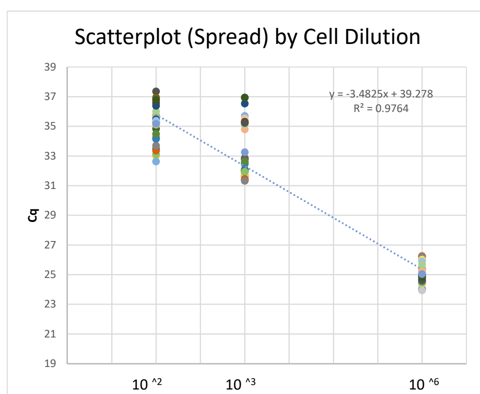




159

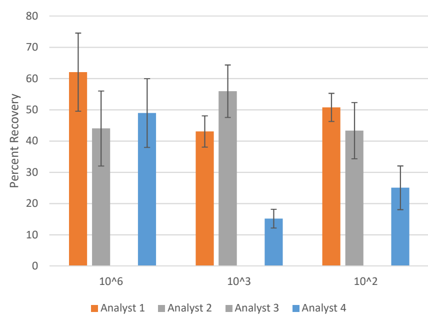
Extraction Efficiency: *L. pneumophila* MIP gene – Cq/200mL

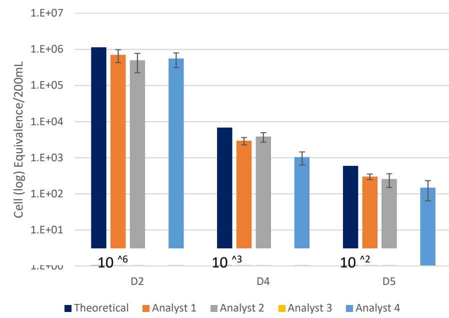




160

Extraction Efficiency: *L. pneumophila* MIP gene - Cell Equivalence/200mL





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Summary Phase II: Percent Recovery

Cq

Gen L	Analyst 1		Analyst 2		Analyst 3		Analyst 4	
	% Recovery	RSD	% Recovery	RSD	% Recovery	RSD	% Recovery	RSD
D2	99.61	2.6	95.59	4.1			99.52	2.2
D4	98.15	1.6	98.39	2.1			94.48	2.2
D5	99.01	1.0	96.76	2.6			93.53	1.7

Lp16S	Analyst 1		Analyst 2		Analyst 3		Analyst 4	
	% Recovery	RSD	% Recovery	RSD	% Recovery	RSD	% Recovery	RSD
D2			92.92	1.44	94.82	3.47	98.51	2.5
D4			96.09	0.64	95.14	1.74	92.57	1.9
D5			95.73	0.82	96.07	2.31	95.84	1.5

MIP A	Analyst 1		Analyst 2		Analyst 3		Analyst 4	
	% Recovery	RSD	% Recovery	RSD	% Recovery	RSD	% Recovery	RSD
D2	97.1	2.2	94.8	3.3			95.8	2.3
D4	96.3	1.2	97.3	1.4			91.9	1.8
D5	97.3	0.7	96.4	2.0			94.3	1.8

Cell Equivalence

Gen L		Analyst 1		Analyst 2		Analyst 3		Analyst 4	
	Theoretical	Recovery	%RDS	Recovery	%RDS	Recovery	%RDS	Recovery	%RDS
D2	952,004	931,099	35	572,635	54			862,986	30
D4	8,833	6,471	27	6,975	38			3,303	48
D5	846	700	20	474	41			216	39

Lp16S		Analyst 1		Analyst 2		Analyst 3		Analyst 4	
	Theoretical	Recovery	%RDS	Recovery	%RDS	Recovery	%RDS	Recovery	%RDS
D2	1,552,857			319,098	50	338,665	26	702,596	31
D4	12,977			4,284	32	3,392	12	2,093	40
D5	1,033			330	36	327	18	326	38

Mip A		Analyst 1		Analyst 2		Analyst 3		Analyst 4	
	Theoretical	Recovery	%RDS	Recovery	%RDS	Recovery	%RDS	Recovery	%RDS
D2	1,138,959	706,657	38	501,326	55			557,594	44
D4	6,852	2,951	23	3,833	29			1,039	47
D5	591	300	18	256	41			148	57

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Phase III: Compete Method-Sensitivity Study

Cell Spikes $\frac{10^5 \text{ Cells or CE}}{200\text{mL}}$ $\frac{10^4 \text{ Cells or CE}}{200\text{mL}}$ $\frac{10^3 \text{ Cells or CE}}{200\text{mL}}$ $\frac{10^2 \text{ Cells or CE}}{200\text{mL}}$ $\frac{10 \text{ Cells or CE}}{200\text{mL}}$

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Phase III: Compete Method-Sensitivity Study

What:

- 6 Concentrations + Method Blank
- 3 spike bottles/concentration (Total: 21 Extractions)
- Each Extraction analyzed in triplicate for each assy.

Who:

- One Internal Lab
- One External Lab

Theoretically Cell Spike: Quantified by spiking 50µL directly into a bead tube.

No Bottle

No membrane filtration

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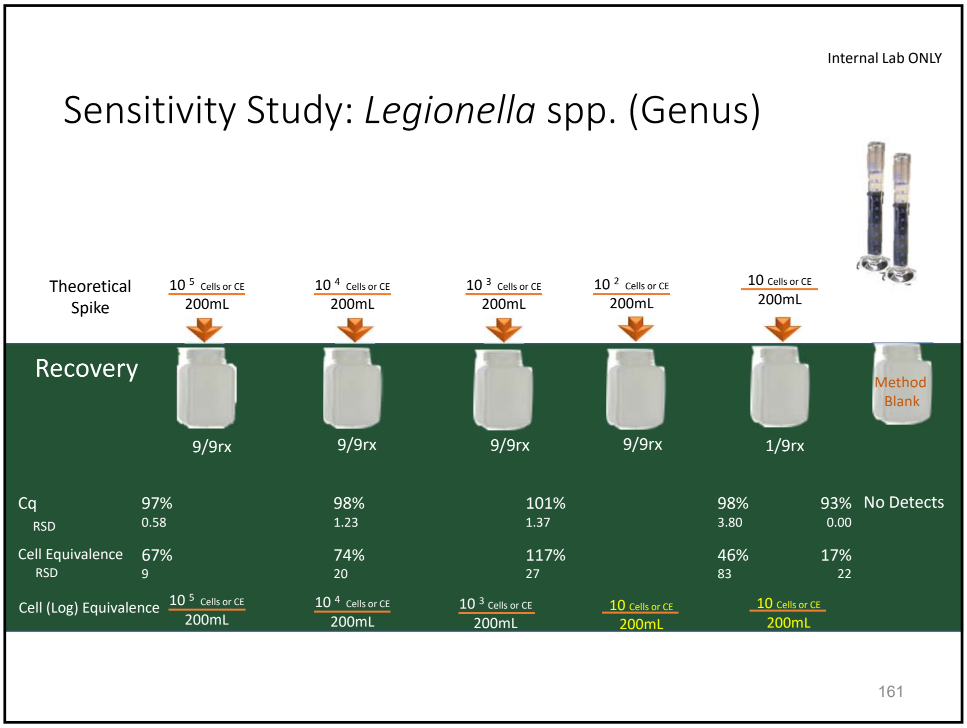
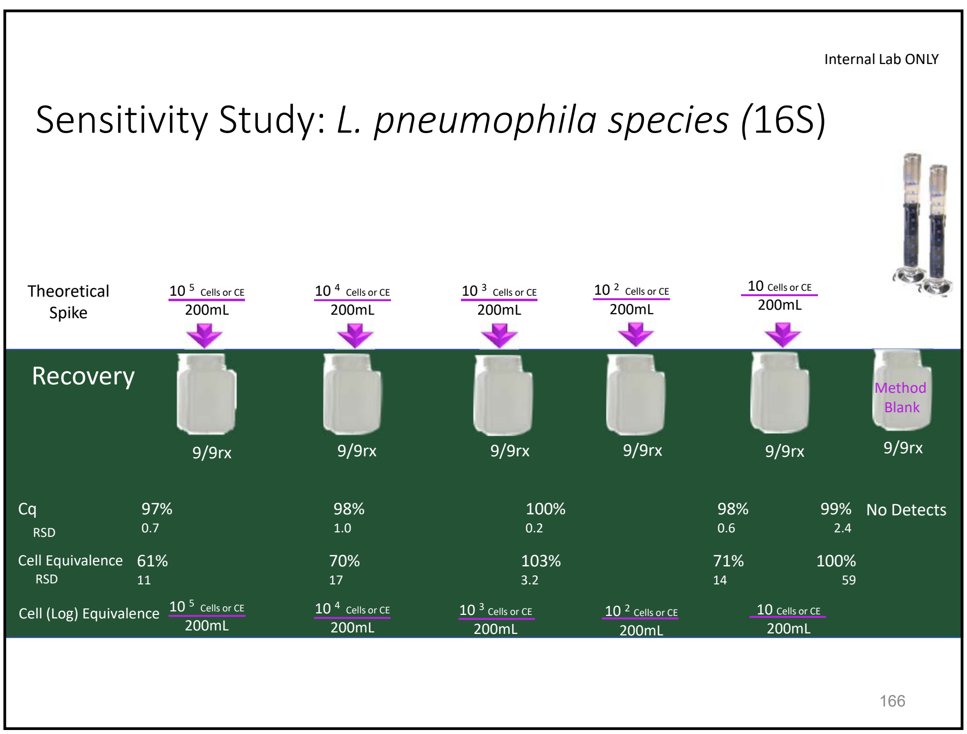

Internal Lab ONLY
Sensitivity Study: *Legionella* spp. (Genus)
Theoretical Spike
10 5 Cells or CE / 200mL
10 4 Cells or CE / 200mL
10 3 Cells or CE / 200mL
10 2 Cells or CE / 200mL
10 Cells or CE / 200mL
Recovery
9/9rx
9/9rx
9/9rx
9/9rx
1/9rx
Method Blank
Cq 97% 98% 101% 98% 93% No Detects
RSD 0.58 1.23 1.37 3.80 0.00
Cell Equivalence 67% 74% 117% 46% 17%
RSD 9 20 27 83 22
Cell (Log) Equivalence
10 5 Cells or CE / 200mL
10 4 Cells or CE / 200mL
10 3 Cells or CE / 200mL
10 Cells or CE / 200mL
10 Cells or CE / 200mL
161


Internal Lab ONLY
Sensitivity Study: *L. pneumophila species* (16S)
Theoretical Spike
10 5 Cells or CE / 200mL
10 4 Cells or CE / 200mL
10 3 Cells or CE / 200mL
10 2 Cells or CE / 200mL
10 Cells or CE / 200mL
Recovery
9/9rx
9/9rx
9/9rx
9/9rx
9/9rx
Method Blank
9/9rx
Cq 97% 98% 100% 98% 99% No Detects
RSD 0.7 1.0 0.2 0.6 2.4
Cell Equivalence 61% 70% 103% 71% 100%
RSD 11 17 3.2 14 59
Cell (Log) Equivalence
10 5 Cells or CE / 200mL
10 4 Cells or CE / 200mL
10 3 Cells or CE / 200mL
10 2 Cells or CE / 200mL
10 Cells or CE / 200mL
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Internal Lab ONLY

Sensitivity Study: *L. pneumophila species* MipA

Theoretical Spike	10^5 Cells or CE / 200mL	10^4 Cells or CE / 200mL	10^3 Cells or CE / 200mL	10^2 Cells or CE / 200mL	10 Cells or CE / 200mL		Method Blank
Recovery	9/9rx	9/9rx	9/9rx	9/9rx	3/9rx		9/9rx
Cq	98%	99%	100%		90%	93%	No Detects
RSD	0.48	1.04	0.55		3.14	0.87	
Cell Equivalence	67%	80%	111%		10%	19%	
RSD	9	21	14		66	22	
Cell (Log) Equivalence	10^5 Cells or CE / 200mL	10^4 Cells or CE / 200mL	10^3 Cells or CE / 200mL	10 Cells or CE / 200mL	10 Cells or CE / 200mL		

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Internal Lab Results ONLY

Summary of Sensitivity Study

Cell Spike per 200 mL

	10^5 Cells or CE	10^4 Cells or CE	10^3 Cells or CE	10^2 Cells or CE	10 Cells or CE	
Cq						
	97%	98%	101%		98%	93%
	97%	98%	100%		98%	99%
	98%	99%	100%		90%	93%
Cell Equivalence						
	67%	74%	117%		46%	17%
	61%	70%	103%		71%	100%
	67%	80%	111%		10%	19%
Cell (Log) Equivalence	10^5 Cells or CE / 200mL	10^4 Cells or CE / 200mL	10^3 Cells or CE / 200mL	10 Cells or CE / 200mL	10 Cells or CE / 200mL	
	10^5 Cells or CE / 200mL	10^4 Cells or CE / 200mL	10^3 Cells or CE / 200mL	10^2 Cells or CE / 200mL	10 Cells or CE / 200mL	
	10^5 Cells or CE / 200mL	10^4 Cells or CE / 200mL	10^3 Cells or CE / 200mL	10 Cells or CE / 200mL	10 Cells or CE / 200mL	

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Status

- All Three Phases have been completed by both internal and external labs.
  - Find another external Lab for Second Lab Verification
- Data has been received from the External Lab for Phase III Sensitivity Study.
  - Analyze data
- Holding time study
- Write Method

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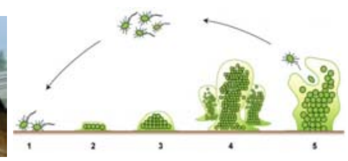
Water Type

- Source/Raw Water
- Potable Water
- Waste Water
- Rain Water
- Sediment

- Biofilm



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- Mr. Frank Greenland
- Rosemarie Read

Dr. Maura Donohue
ORD/NERL/EMMD/PHCB

Thank You!

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Questions

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Detection Methods for Mycobacteria

Stacy Pfaller

Ohio River and Downtown Cincinnati, OH

Office of Research and Development
National Exposure Research Laboratory, Systems Exposure Division

EPA United States Environmental Protection Agency

Background

- Waterborne illness caused by nontuberculous mycobacteria (NTM) cost nearly $1.8 B for in-patient and out-patient treatment in 2010 ([a]Thomson et al, 2015).
- Pulmonary NTM infections account for almost half of all NTM hospitalizations in the US, and are typically caused by *Mycobacterium avium* (MA) and *M. intracellulare (MI)*
- In addition to pulmonary infections, can cause skin, soft tissue, lymph node, systemic infections, among others
- Primary source of human exposure: **WATER**
- **CCL's 1 and 2:** *Mycobacterium avium* Complex (MAC)
- **CCL's 3 and 4:** *M. avium*

  4 subspecies: *M. avium* subsp. *hominissuis*
  M. avium subsp. *avium*
  M. avium subsp. *silvaticum*
  M. avium subsp. *paratuberculosis*

[a]Thomson et al (2015) Ann Am Thorac Soc, Vol 12:1425–1427

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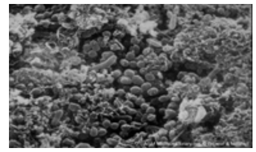

Culture and Molecular Methods

- **Culture Method**

  Standard Methods for the Examination of Water and Wastewater[a] section 9260M with modifications described in Covert et al (1999) Appl Environ Microbiol 65:2492-2496

- **Quantitative PCR (qPCR)**

  Beumer et al, (2010) Appl Environ Microbiol 76:7367-7370 and Figure S1, Supplemental Material http://aem.asm.org/content/76/21/7367/suppl/DC1
  Chern et al, (2015) J Wat Health 13.1:131-139

[a]Eaton, A. D., L. S. Clesceri, E. W. Rice, and A. E. Greenberg (ed.). 2005. Standard methods for the examination of water and wastewater, 21st ed. American Public Health Association, Washington, DC.

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Sample Collection

- Sample collection is identical for both culture and qPCR
  - Bulk Water: collected in 1L sterile polypropylene bottles, NO preservative ($Na_2S_2O_3$), according to sections 9060A and B of Standard Methods for the Examination of Water and Wastewater[a]
- Samples transported back to lab on ice, stored at 4 ° C until processing, within 48 hours

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Culture method

- ❖1L water split into replicate aliquots (vol. depends on sample type)

- ❖Samples filtered through 0.45 um pore-size, 47 mm black-grid, cellulose ester filter by vacuum filtration, washing the filter with sterile deionized water, filter aseptically transferred to Middlebrook 7H10 agar containing 500 mg L^{-1} cycloheximide

- ❖Plates are incubated a **minimum 8 weeks** at 37 ° C and inspected weekly for growth

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QC samples for Culture method

❖Sterile medium negative control

- Performed when medium is made, in advance of samples arriving
- Incubation of un-inoculated medium to ensure sterility

❖Method blank negative control

- Sterile deionized water filtered processed at the same time in the same way as unknowns

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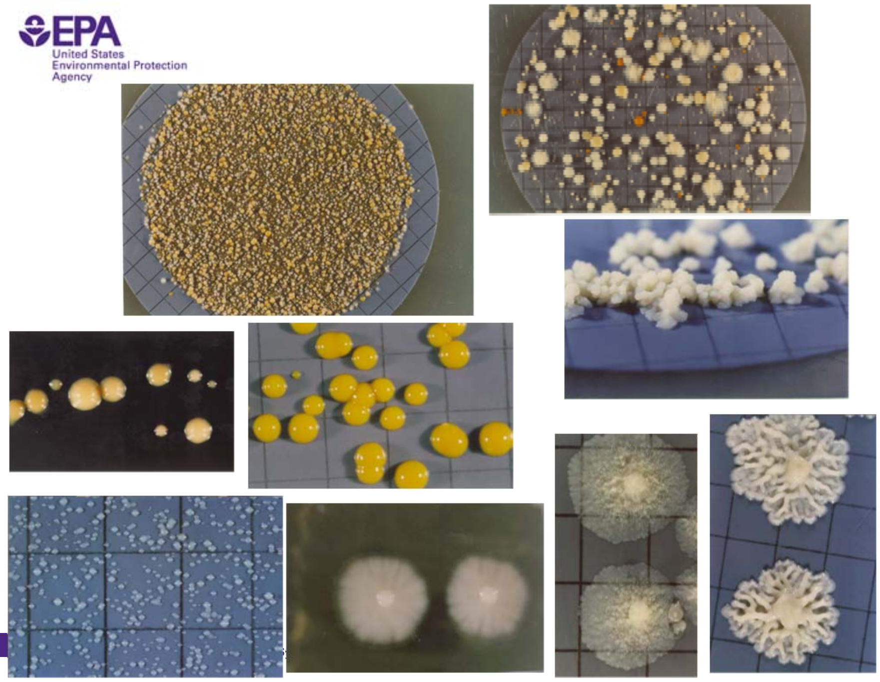

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Benefits of Culture Method

- ❖Many species of NTM can grow on medium
- ❖Live only detection
- ❖Obtain a culture collection for future characterization
  - •Genotype
  - •Virulence genes

Drawbacks of Culture Method

- ❖Method has not been characterized for specificity or sensitivity
- • ***medium is not selective for mycobacteria***
- ❖Cetyl pyridinium chloride (CPC) disinfection may reduce recovery of target by 70%

  (Personal communication: Terry Covert)
- ❖Every colony is an unknown in need of identification
- ❖Only a subset of colonies can be chosen for identification
- • ***method is not quantitative***
- ❖Months to years before results are obtained
- ❖Performs poorly on biofilm

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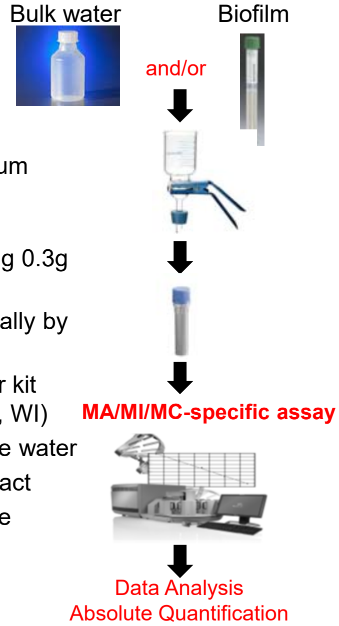
qPCR Method



- 1 L water (per target assay) or biofilm slurry is vacuum filtered through 47.0 mm, 0.45 um polycarbonate membrane
- Membrane rolled and placed in 2.0ml tube containing 0.3g glass beads and buffer
- Microorganisms trapped on membrane lysed physically by bead beating
- DNA from crude lysate extracted using WaterMaster kit reagents from (EpiCenter Biotechnologies, Madison, WI)
- DNA resuspended in sterile, molecular biology-grade water
- Three replicate qPCR reactions analyzed/ DNA extract
  - Two replicates must be positive for a sample to be considered positive

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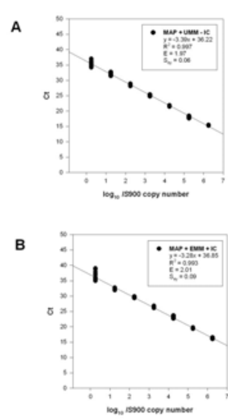
Absolute Quantification from Master Standard Curves

- Generated from six independents series of 10-fold serial dilutions of purified genomic DNA from ATCC Type strains of MA, MI/MC
- Each dilution series contains eight standards, ranging in concentration from 10^6 target copies to 1 copy, run in triplicate = 18 measurements/ standard
- C_T measurements plotted against log target number and analyzed by linear regression to generate line equation
- Target number in unknown sample estimated from line equation



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QC Samples for qPCR

- **Method Blank** (negative control)
  - Sterile molecular biology grade water filtered and processed at the same time in the same way as unknowns
- **Extraction Control**
  - Sterile filter processed at the same time in the same way as unknowns
- **Standards** (positive control)
  - Purified genomic DNA from target, serially diluted
- **No Template Control** (negative control)
  - Sterile molecular biology grade water added to qPCR reaction instead of DNA extract
- **Internal positive Control** (IC)
  - Commercially available kit (TaqMan Exogenous Internal Positive Control Kit, Life Technologies, Carlsbad, CA)

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Benefits of qPCR Method

- Assays are specific for MA and MI/MC
- Quantitative
- Time to results = 3 days

Drawbacks of qPCR Method

- Assays are specific for MA, MI/MC only
- Cannot distinguish between live and dead organisms though studies have demonstrated that DNA contained within chlorine disinfected cells does not typically persist in water with a chlorine residual

  Page et al, 2010, Appl Environ Microbiol, 29:2946-2954
  Sen et al, 2010, Current Microbiol, 62:727-732

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Mycobacterium qPCR Method Verification

- Started December 2014 in coordination with Maura Donohue and the *Legionella* qPCR method verification
- Two *Mycobacterium* assays
  - *M. avium*
  - *M. intracellulare/chimaera*
- Verification performed in three phases
  - Phase I: Characterizing LOD, LOQ from generation of DNA standard curves (First performed on LifeTech StepOne instrument, repeated on new LifeTech Quant 6 Studio) = complete
  - Phase II: Characterizing target extraction efficiency = needs repeating

    Options:
    - Bioballs containing known DNA concentrations
    - Known DNA concentrations spiked on filters
  - Phase III: Characterizing method sensitivity = not complete

Characteristics of qPCR assays for drinking water and biofilm

qPCR assay	Target (copies/ genome)	E¶ Amplification Efficiency	LOD‡ Targets/qPCR reaction	LOQ† Targets/qPCR reaction	Specificity £	Sensitivity § Drinking Water
M. avium	16S rDNA (1)	103%	10	10	100%	Not determined
M. intracellulare/ chimaera	16S rDNA (1)	92%	10	10	100%	Not determined

¶**Amplification Efficiency** = -1 + $10^{(-1/slope)}$. Acceptable range = 90 – 110%.
‡**LOD** = Limit of detection = lowest copy number/assay giving C_T < 40 in 6/6 independent assays.
†**LOQ** = Limit of quantification = lowest copy number/assay yielding a coefficient of variation < 25%.
£**Specificity** = Number of target testing positive/total number targets tested x 100.
§ **Sensitivity** = lowest copy number detected when spiking serial dilutions of known cell quantities into actual tap water samples, processed as described.

Additional qPCR Methods

- The literature describes many qPCR assays for targeting various taxonomic levels within the genus *Mycobacterium*
  - ***Mycobacterium* Genus-specific assays**
    - Bruijnesteijn van Coppenraet, E.S., Lindeboom, J.A., Prins, J.M.,Peeters, M.F., Claas, E.C.J. and Kuijper, E.J. (2004) J Clin Microbiol 42:2644-2660.
    - Radomski, N., Lucas, F.S., Moilleron, R., Cambau, E., Haenn, S., Moulin, L. (2010) Appl Environ Microbiol 76:7348-7351.
  - ***Mycobacterium* species-specific assays**
    - *M. avium*: Feazel, L.M., Baumgartner, L.K., Peterson, K. L., Frank, D.N., Kirk Harris, J., Pace, N.R. (2009) PNAS 106:16393-16399
    - *M. tuberculosis, M. avium, M. intracellulare, M. kansasii, M. abscessus, M. massilense,* and *M. fortuitum*: Kim, K.I., Lee, H., Lee, M.-K., Lee, S.-A., Shim, T.-S., Lim, S.Y., Koh, W.-J., Yim, J.-J., Munkhtsetseg, B., Kim, W., Chung, S.-I., Kook, Y.-H., Kim, B.-J. (2010) J Clin Microbiol 48:3073-3080.
    - Eighteen *Mycobacterium* species: Lim, S.Y., Kim, B.-J., Lee, M.-K., Kim, I.K., Lett Appl Microbiol (2008) 46:101-106.

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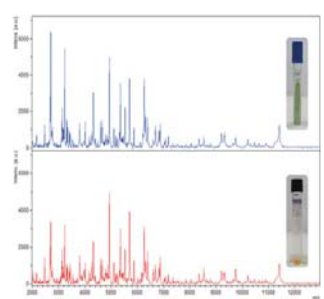
Identification of Isolates using Matrix Assisted Laser Desorption/ Ionization (MALDI) Protein Profiles

- Performed on purified culture isolates
- More rapid than DNA sequencing methods
- Two systems for bacterial identification:
  - Bruker MALDI Biotyper and *Mycobacterium* database: 164 species with unique profiles
  - Biomérieux Vitek MS and V3 database for molds, *Nocardia*, and *Mycobacterium*



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Best Method for *Mycobacterium* Detection for Finished Drinking Water?

- qPCR performs regardless of sample matrix (water or biofilm) and volumes up to 10 L are easily and rapidly analyzed
- Culture does not perform on microbiologically complex samples (water and biofilm) but does perform on samples where microbiological water quality is high (treated water before distribution)

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Acknowledgments

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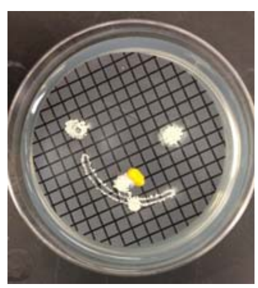

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Questions

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Open Forum and Discussion

Brenda Parris

U.S. EPA
Office of Ground Water and Drinking Water
Technical Support Center

Webinar Participant Questions

- Click on "+" next to "Questions" in the control panel (Figure 1) to submit questions/comments
  - You may need to unhide the control panel to ask a question (Figure 2)
- Type a question in the box; click send (Figure 3)

Figure 1

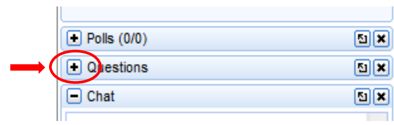
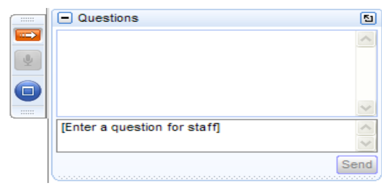


Figure 2

Figure 3



If You Have Questions Following This Meeting/Webinar

- Analytical Methods for Drinking Water Homepage:
  - https://www.epa.gov/dwanalyticalmethods/analytical-methods-developed-epa-analysis-unregulated-contaminants
- Methods
  - Presenters
- Webinar
  - methodsdevelopment@cadmusgroup.com

Break  **(15 minutes)**

Open Forum and Discussion

Closing Remarks

Brenda Parris, U.S. EPA

Office of Ground Water and Drinking Water
Technical Support Center

Abbreviations and Acronyms

- **CCC** – Continuing Calibration Check
- **CCL** – Contaminant Candidate List
- **CDC** – Centers for Disease Control and Prevention
- **CIS** – Contaminant Information Sheet
- **Cq** – Quantitation Cycle
- **DAI** – Direct Aqueous Injection
- **DIMP** – Diisopropyl methylphosphonate
- **DL** – Detection Limit
- **DNA** – Deoxyribonucleic Acid

Abbreviations and Acronyms

- **dNTP** – Deoxyribonucleotide Triphosphate
- **DQO** – Data Quality Objective
- **DVB** – Divinylbenzene
- **ESA** – Ethane Sulfonic Acid
- **ESI** – Electrospray Ionization
- **FR** – Federal Register
- **GC** – Gas Chromatography
- **GC** – Gas Chromatography
- **GenX** – Perfluoro-2-propoxypropanoic acid

Abbreviations and Acronyms

- **HLB** – Hydrophilic Lipophilic Balanced
- **HRL** – Health Reference Level
- **HVAC** – Heating, Venting, and Air Conditioning
- **i.d.** – Internal Diameter
- **IDC** – Initial Demonstration of Capability
- **IS** – Internal Standard
- **ISO** –International Organization for Standardization
- **IUPAC** – International Union of Pure and Applied Chemistry
- **LC** – Liquid Chromatography

Abbreviations and Acronyms

- **LCMRL** – Lowest Concentration Minimum Reporting Level
- **LFB** – Laboratory Fortified Blank
- **LFSM** – Laboratory Fortified Sample Matrix
- **LFSMD** – Laboratory Fortified Sample Matrix Duplicate
- **LLE** – Liquid Liquid Extraction
- **LOD** – Limit of detection
- **LOQ** – Limit of quantification
- **LRB** – Laboratory Reagent Blank
- **MCLG** – Maximum Contaminant Level Goal

Abbreviations and Acronyms

- **MDL** – Method Detection Limit
- **MRL** – Minimum Reporting Level
- **MRM** – Multiple Reaction Monitoring
- **MS** – Mass Spectrometry
- **MS/MS** –Tandem Mass Spectrometry
- **NCOD** – National Contaminant Occurrence Database
- **NDEA** – N-Nitrosodiethylamine
- **NDMA** – N-Nitrosodimethylamine
- **NDPA** – N-Nitrosodipropylamine

Abbreviations and Acronyms

- **NP** – Nonylphenol
- **NPDWRs** – National Primary Drinking Water Regulations
- **NPYR** – N-Nitrosopyrrolidine
- **NTM** – Nontuberculous Mycobacteria
- **OA** – Oxanilic Acid
- **PAH** – Polycyclic Aromatic Hydrocarbons
- **PCB** – Polychlorinated Biphenyl
- **PCR** –Polymerase Chain Reaction
- **PFAS** – Per- and Polyfluoroalkyl Substances

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Abbreviations and Acronyms

- **PFOA** – Perfluorooctanoic Acid
- **PFOS** – Perfluorooctane Sulfonic Acid
- **PWS** – Public Water System
- **QC** – Quality Control
- **QCS** – Quality Control Sample
- **qPCR** – Quantitative Polymerase Chain Reaction
- **RNA** – Ribonucleic Acid
- **RSD** – Relative Standard Deviation
- **SDWA** – Safe Drinking Water Act

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Abbreviations and Acronyms

- **SIM** – Select Ion Monitoring
- **SPE** – Solid Phase Extraction
- **SUR** – Surrogate Standard
- **SVOC** – Semivolatile Organic Compound
- **TPTH** – Triphenyltin hydroxide
- **TOC** – Total Organic Carbon
- **TT** – Treatment Technique
- **UCMR** – Unregulated Contaminant Monitoring Rule
- **UPLC** – Ultra Performance Liquid Chromatography

Abbreviations and Acronyms

- **UV** – Ultraviolet Light
- **VOC** – Volatile Organic Compound
- **WAX SPE** – Weak Anion Exchange Solid Phase Extraction