Per- and Polyflouroalkyl Substances (PFAS) Laboratory Testing Primer for State Drinking Water Programs and Public Water Systems

Association of State Drinking Water Administrators

This document provides guidance on how state drinking water programs can work with laboratories to test per- and polyfluoroalkyl substances (PFAS) in drinking water samples collected from public water systems. The document provides information on:

1) Selecting an analytical method;
2) Finding a qualified laboratory;
3) Specifying a PFAS list and the form that each PFAS needs to be reported in;
4) Specifying reporting limits;
5) Sample collection procedures;
6) Technical issues that cause variability in testing results;
7) Interpreting results; and
8) USEPA's ongoing work to develop new analytical methods

Topic 1: Selecting an Analytical Method

Different laboratories provide different analytical options for measuring PFAS in drinking water. These include:

1) EPA Method 537 Rev. 1.1 - Determination of Selected Perfluorinated Alkyl Acids in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)(2009). Note the "Rev 1.1" designation indicates the method was edited to clarify procedures or make editorial revisions from the originally published method.
2) EPA Method 537.1 Rev. 2 - Determination of Selected Per- and Polyflourinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) (2020) (Method 537.1 Revision 2.0 is an editorial update to Method 537.1 2018). Note the "Rev 2" designation indicates the method was edited to clarify procedures or make editorial revisions from the original method that was released in November 2018.
3) EPA Method 533: Determination of PFAS in Drinking Water by Isotope Dilution Anion Exchange Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (November 2019).
4) Determination of PFAS using isotope dilution via proprietary methods developed by individual laboratories. These method names will vary from laboratory to laboratory. Although these analytical methods are completely different from EPA Method 537 Rev. 1.1 which does not use isotope dilution, some laboratories refer to their isotope dilution method as "EPA 537-modified." EPA has not validated proprietary or modified methods.

Each of the analytical methods listed above can provide reliable results if an accredited/certified laboratory is used. The methods can also generally achieve reporting limits of 2-8 nanograms per liter (ng/L) or parts-per-trillion (ppt) or lower. The achievable reporting limits should be verified with the laboratory prior to sample collection.

It is recommended that drinking water systems use an EPA-validated method. There may be circumstances though when a proprietary analytical method is desirable because a specific PFAS is not included in an EPA method. Water systems should then follow their state drinking water program's requirements or guidance.

Method 537 tests for 14 PFAS and Method 537.1 tests for 18 PFAS, inclusive of these 14 PFAS, plus two PFOA alternatives (HFPO-DA, a GenX process chemical, and ADONA) and a Chinese PFOS alternative with two forms (9Cl-PF3ONS and 11Cl-PF3Ouds). Quality control measures in these methods include adding surrogates prior to solid-phase extraction (SPE) to assess sample preparation analyte loss and adding internal standards to the final sample extract to assess instrument performance. Methods 537 and 537.1 do not include smaller chain PFAS (C4 PFAS such as PFBA) because the methods generally perform poorly for these compounds. It is important to consider that small-chain PFAS are generally the first compounds to break through water treatment processes.

Method 533 tests for 25 PFAS (inclusive of 14 of the 18 PFAS in EPA 537.1, plus 11 more PFAS). There are two primary procedural differences between the methods. Method 533 incorporates the use of extracted internal standards as part of an isotopic dilution quantification approach whereas Method 537.1 does not. The use of the isotope dilution technique reduces the overall uncertainty associated with the analysis. Method 533 also uses a weak anion exchange (WAX) solid phase extraction (SPE) cartridge instead of the polystyrene divinylbenzene (SDVB) used in Method 537.1. The use of the WAX SPE cartridge in Method 533 allows for additional PFAS compounds to be determined, particularly short chain PFAS not amenable to Method 537.1 analysis. [Source: ALPHA Analytical]

The list of analytes included in Methods 537, 537.1 and 533 are shown in the table on the next page.

Field Reagent Blanks and EPA Method 537, 537.1 and 533

It is important to note that all three EPA methods require water systems to collect a field reagent blank (FRB) at each sampling location. This quality control requirement addresses cross sample collection contamination concerns due to the ubiquity of these compounds in the environment and method reporting limits at highly-sensitive sub parts-per-trillion (ppt) levels. FRBs are used to assess field contamination from materials such as Tyvek personal protective equipment (PPE) that might include PFAS residues. Some states have found that with sound sampling protocols, PFAS are not routinely detected in public water system FRBs because the sample collection process does not regularly introduce contaminants into the water sample. Other states have had different experiences and have seen a number of FRB test results with detectable PFAS levels.

Some state drinking water programs specifically waive the one FRB per water sample requirement and some state drinking water programs require an FRB with each water sample. FRBs submitted to a laboratory sometimes may only be analyzed if PFAS are detected in the corresponding water sample. When holding time is a concern, some laboratories require both the water sample and corresponding FRB to be analyzed. It is important to be aware that a laboratory may charge for FRBs, increasing analysis, shipping, and handling costs.

EPA 537.1 Field Reagent Blank bottle contents clarification: At the laboratory, fill a field blank sample bottle with reagent water, then seal, and ship to the sampling site along with the sample bottles. For each FRB shipped, a second FRB bottle containing only the Trizma® preservative must also be shipped. At the sampling site, the sampler must open the shipped FRB and pour the reagent water into the shipped sample bottle containing only Trizma®, seal and label this bottle as the FRB.

Comparing EPA Method 537, EPA Method 537.1 and EPA Method 533 Analytes

	Analyte	Abbreviation	CASRN	Method 533	Method 537	Method 537.1
1	11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9	X		X
2	9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	756426-58-1	X		X
3	4,8-Dioxa-3H-perfluorononanoic acid	ADONA	919005-14-4	X		X
4	Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6	X		X
5	Perfluorobutanesulfonic acid	PFBS	375-73-5	X	X	X
6	Perfluorodecanoic acid	PFDA	335-76-2	X	X	X
7	Perfluorododecanoic acid	PFDoA	307-55-1	X	X	X
8	Perfluoroheptanoic acid	PFHpA	375-85-9	X	X	X
9	Perfluorohexanoic acid	PFHxA	307-24-4	X	X	X
10	Perfluorohexanesulfonic acid	PFHxS	355-46-4	X	X	X
11	Perfluorononanoic acid	PFNA	375-95-1	X	X	X
12	Perfluorooctanoic acid	PFOA	335-67-1	X	X	X
13	Perfluorooctanesulfonic acid	PFOS	1763-23-1	X	X	X
14	Perfluoroundecanoic acid	PFUnA	2058-94-8	X	X	X
15	1H,1H, 2H, 2H-Perfluorohexane sulfonic acid	4:2FTS	757124-72-4	X		
16	1H,1H, 2H, 2H-Perfluorooctane sulfonic acid	6:2FTS	27619-97-2	X		
17	1H,1H, 2H, 2H-Perfluorodecane sulfonic acid	8:2FTS	39108-34-4	X		
18	Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	151772-58-6	X		
19	Perfluorobutanoic acid	PFBA	375-22-4	X		
20	Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	113507-82-7	X		
21	Perfluoroheptanesulfonic acid	PFHpS	375-92-8	X		
22	Perfluoro-4-methoxybutanoic acid	PFMBA	863090-89-5	X		
23	Perfluoro-3-methoxypropanoic acid	PFMPA	377-73-1	X		
24	Perfluoropentanoic acid	PFPeA	2706-90-3	X		
25	Perfluoropentanesulfonic acid	PFPeS	2706-91-4	X		
26	N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	2991-50-6		X	X
27	N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	2355-31-9		X	X
28	Perfluorotetradecanoic acid	PFTA	376-06-7		X	X
29	Perfluorotridecanoic acid	PFTrDA	72629-94-8		X	X

Topic 2: Finding a Qualified Laboratory

A water system should check with their state drinking water program to see if their state has established a list of approved or accredited laboratories that can analyze drinking water for PFAS. If the state has not established a list, water systems can identify qualified laboratories by using the following resources:

1) National Environmental Laboratory Accreditation Program - select a common PFAS chemical such as perfluorooctanoic acid (PFOA) under the "Analyte" filter or enter "537, 537.1 or "533" in the "method" filter.
2) The Department of Defense - select EPA 537 or EPA 533 under the "Method" drop down menu.
3) Find state-certified labs.

Laboratories that routinely work with water systems may already have or can establish subcontracts with accredited laboratories.

Topic 3: Which PFAS Chemicals Should Be Analyzed?

A water system should check with their state drinking water program to see if their state has established a list of PFAS that should analyzed. There are thousands of PFAS. Commercial laboratories can typically test for approximately 6 to 40 PFAS. A laboratory may offer multiple options on the number of PFAS that will be reported. Generally, the cost per sample increases when more PFAS are measured. However, the cost increase is often relatively minor and is not usually proportional to the increased number of compounds.

Laboratory analyses that include a longer list of PFAS may provide more information on the PFAS source(s) that could be impacting the drinking water. While there may be no health guidance at this time for the majority of PFAS, future guidance or toxicity information may inform historical test results. Six PFAS (PFOS, PFOA, PFNA, PFHxS, PFHpA and PFBS) were included in USEPA's Third Unregulated Contaminant Monitoring Rule (UCMR3) and for this reason, at a minimum, states and water systems should always consider including these six PFAS in their laboratory-requested analyses. PFAS included in the UCMR3 sampling were selected based on current research pertaining to potential occurrence and health risk factors. Three additional PFAS (PFBA, PFPeA and PFHxA) have been regularly detected in drinking water systems throughout the nation and it is suggested that these PFAS be included along with the six UCMR3 PFAS (listed above) in the PFAS analysis.

The PFAS analyses described above may only quantify a fraction of the PFAS, PFAS precursors, and/or total organic fluorine contamination present in drinking water. Some water systems have worked with research laboratories to more fully identify and quantify the potential for other highly-fluorinated chemicals in drinking water. This work generally goes beyond any regulatory guidance or requirements. More information on these testing methods can be found in the Interstate Technology Regulatory Council (ITRC) factsheet, "Site Characterization Considerations, Sampling Precautions, and Laboratory Analytical Methods for PFAS (April 2020)."

Laboratories Must Report PFAS Correctly

Laboratories need to ensure analytical reports, electronic data deliverables (EDDs), and laboratory-developed reports communicate all EPA method-required results in the proper format. These should reflect the exact chemical name and CAS number specified in the EPA methods. Please see the related "Technical Bulletin to Laboratories Reporting PFAS Analysis…" to ensure accurate reporting.

State drinking water programs have identified numerous instances where laboratory reports, EDDs and analytical reports produced by laboratories that are subcontracting PFAS testing services to other laboratories have used the anionic and acid form names incorrectly and sometimes interchangeably (for example, perfluorooctane sulfonate and perfluorooctane sulfonic acid are not the same compound and their names cannot be used interchangeably). Adding to the confusion, different PFAS forms can share a common acronym or conversely, the same PFAS are named with different acronyms. The inconsistent use of PFAS names and associated CAS numbers is problematic for multiple reasons including:

1) PFAS results are being reported in a form that is inconsistent with the requirements of EPA Methods 533, 537, and 537.1;
2) PFAS with different names and CAS numbers are different in terms of physical and chemical properties, so it is important to know which form is being measured and reported;
3) The name of a PFAS often does not match test report CAS numbers and the subcontract laboratory-generated EDDs;
4) PFAS analytical results are being reported using chemical forms that are different than state drinking water standards or guidelines; and
5) The reporting of PFAS using multiple chemical names and CAS numbers create a significant database management challenge. This often results in inaccurate query results because multiple forms of similar PFAS are within the same dataset.

Topic 4: What Reporting Limits Should Be Required?

Laboratory should use analytical methods with reporting limits (RL) of at least 2-4 ng/L (or ppt). Many commercial labs are achieving RLs of less than 1 ng/L (or ppt). PFAS health studies are rapidly evolving and are looking at low-level exposure. Some states have determined that PFAS health advisory concentrations in drinking water should be based on the additive effect of PFAS (i.e., combined rather than individual PFAS). Obtaining water quality results with lower RLs will improve the data's utility if a new health guidance or standard is developed based on the additive effects of PFAS.

It is important to understand the difference between an RL and a detection limit (DL). A RL is the limit of quantitation in which the concentration of a contaminant can be reliably quantified using appropriate low calibration standards. In contrast, the method DL should be lower than the RL, and is the level below which the detected concentrations are unreliable. Results that fall into this range should be qualified by carrying a "J" (NELAP) qualifier/flag.

Topic 5: Technical Issues that Cause Variability in Testing Results

Sample Results Prior to September 2016 May Be Under-Reported

Many PFAS can be present as linear and branched isomers. PFAS with the same chemical formula (i.e., same molecular weight) but different atomic structures are isomers and can elute from analytical instrumentation at different times. Initially, DuPont produced PFOA using telomerization processes that resulted in a completely linear isomer form of PFOA. However, 3M later produced PFAS using electrochemical fluorination processes that resulted in approximately 78% linear and 22% branched isomers for PFOA. [Source: PAGE 538 https://www.atsdr.cdc.gov/toxprofiles/tp200-c5.pdf]

In September 2016, USEPA issued a technical advisory to laboratories using Method 537 stating the concentration of both the linear and branched isomers of PFOA need to be quantified and added to determine the total PFOA concentration. Prior to this technical advisory, USEPA Method 537 did not stipulate this requirement and some laboratories only reported PFOA in the linear isomers form, while others were reporting PFOA by combining linear and branched isomers. This means some PFOA analyses completed prior to September 2016 may be underreported by as much as 30%.

Expected Accuracy of Testing Results and Common Biases

EPA Methods 537, 537.1 and 533 establish accuracy limits +/- 30%-50% in the analytical method. In a 2016-2017 proficiency testing program that included four commercial laboratories contracted by the New Hampshire Department of Environmental Services, USEPA established accuracy acceptance limits of 50% to 150% of the expected value. This means the expected accuracy of Method 537 series or isotope dilution PFAS analyses is +/-50%. The proficiency testing program results determined: 1) Testing results for split samples sent to the laboratories were generally similar; 2) Testing results for split samples analyzed using Method 537 and proprietary isotope dilution methods were similar; 3) Results were generally accurate within 20% of the expected value but were almost always under-reported from the expected value; and 4) Significant over- or under-reporting of PFAS concentrations occasionally occurred.

Existing PFAS analytical methods use an "extraction" process to isolate the PFAS in a drinking water sample so it can be measured by the instrument. This can be accomplished by using solvents to concentrate (or absorbent material to capture) PFAS. Extraction methods are designed to minimize losses, but generally are not able to completely extract all PFAS present in a water sample. Under-reporting of PFAS results is likely associated with losses that occur during the analytical extraction process. Proficiency testing for other chemicals such as pesticides that rely on extraction processes as part of the sample preparation also show similar under-reporting outcomes.

Certified Standards Are Source of Variability

Laboratories purchase certified standards for PFAS analytes from different vendors, but these standards have been shown to vary by as much as 20%. Additionally, PFAS standards that contain both branched and linear isomers of some PFAS are not available, and laboratories have to estimate the concentration of a compound in the branched form using the linear standard, which results in variations of the reported concentrations.

Laboratory Analyses May Vary

Laboratories using the same validated EPA methods may produce results with some variability when compared to one another. One source of this variability may be due to how laboratories integrate chromatographic peaks for the branched isomers. Several peaks may be present under a multiple reaction monitoring (MRM) chromatogram. This additional step to integrate these peaks may add variability when quantifying the branched isomer concentrations. In contrast, linear isomers only have a single peak and do not require this additional step.

Topic 6: Sample Collection Procedures

PFAS reporting limits are in the ng/L (or ppt), not the µg/L (or parts-per-billion (ppb)) and mg/L (or parts-per-million (ppm)) levels typically reported for drinking water analyses. Additionally, numerous PFAS sources may be at any given location due to their widespread domestic, commercial,

and institutional uses, increasing the potential for PFAS cross-contamination into a drinking water sample during the sample collection process. While in most instances certified drinking water operators are qualified to sample water systems for PFAS, additional training may be required to ensure they understand proper PFAS sampling collection procedures.

Category	Prohibited Items/Actions that Could Introduce PFAS Sample Contamination	Allowable Items
Pumps and Tubing	Teflon® and other fluoropolymer containing materials	High-density polyethylene (HDPE), low density polyethylene (LDPE) or silicone tubing
Sample Container Storage	Containers should not come in to contact with carpeting or upholstery inside buildings or vehicles	Containers should be stored in a zip- lock bag and transported in coolers.
Stacked Glassware	Foil should not be used as a layer between stacked glassware	Plain paper
Field Documentation	Waterproof/treated paper or field books, plastic clipboards, non-Sharpie® markers, Post-It® and other adhesive paper products	Plain Paper, metal clipboard, Sharpies®, pens
Clothing	Clothing or boots made of or with Gore- Tex™ or other synthetic water resistant and/or stain resistant materials, Tyvek® material	Synthetic or cotton material, previously laundered clothing (preferably previously washed greater than six times) without the use of fabric softeners
Personal Care Products (for day of sample collection)	Cosmetics, moisturizers, hand cream and other related products	Sunscreens: Alba Organics Natural Yes to Cucumbers Aubrey Organics Jason Natural Sun Block Kiss My Face Baby-safe sunscreens ('free' or 'natural) Insect Repellents: Jason Natural Quit Bugging Me Repel Lemon Eucalyptus Herbal Armor California Baby Natural Bug Spray BabyGanics Sunscreen and Insect Repellents: Avon Skin So Soft Bug Guard-SPF 30
Food and Beverage	Pre-packaged food, fast food wrappers or containers	

Depending on the sampling objective, the water sample may be collected post-treatment at the distribution system's entry point or at a raw water sampling tap. Existing water treatment systems generally would not affect the water's PFAS concentration unless carbon treatment (e.g., charcoal filter) is in use. Carbon treatment reduces the amount of PFAS in water. If a single entry-point to the

distribution system receives an irregular blend of water from multiple sources of water, a sampling strategy that characterizes the PFAS concentration in each potential blend or from each source should be used. The plumbing associated with each sampling tap should be examined to ensure Teflon tubing and/or fittings are not in use. The sampling process typically includes running water through the sampling tap for two to five minutes, washing hands and using a new pair of nitrile gloves with each sample. PFAS samples should be collected first. The table above identifies some categories of items that could introduce PFAS contamination into the sample during the collection process and appropriate alternatives that can be used to avoid inadvertent sampling contamination.

Trip blank and field reagent blank (FRB) samples should be collected as required by the state, laboratory, and/or method. A trip blank consists of a laboratory-provided bottle of water verified to contain no detectable PFAS levels. The bottle travels with the PFAS sample containers from the laboratory to the sampling location and back to the laboratory with the collected water samples. The trip blank is analyzed to assess the potential for PFAS contamination introduced during the shipping and storage of the sampling containers.

An FRB also consists of a laboratory-provided bottle of water verified to contain no detectable PFAS levels. The bottle travels from the laboratory to the location of the sampling. At the location where drinking water samples are collected from a sampling tab, the FRB bottle contents are transferred into a sampling bottle after the sampler washes his/her hands and wears a new pair of nitrile gloves. The FRB is analyzed to assess the potential for PFAS contamination introduced during the sampling process.

If there are detections of PFAS in the trip blanks or FRBs, it is important to assess if the same PFAS were detected in laboratory blanks, which are generally included in all laboratory analyses at no additional cost. If PFAS are detected in laboratory blanks, then PFAS detections in the FRBs or trip blanks are not likely associated with contamination from the shipping, storage or sampling process. Accredited laboratories qualify drinking sampling results with a letter flag next to the numeric result when PFAS has been detected in the laboratory blank.

Topic 7: Interpreting Results

Results are reported by laboratories on printed documents and/or electronic data deliverables (EDDs). USEPA has established a health advisory for two PFAS combined, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) at 70 ng/L (or ppt). If the total concentration of PFOA and PFOS added together exceeds 70 ng/L, then USEPA's health advisory is exceeded. USEPA's health advisory is not a legally enforceable federal standard, but some states have adopted the health advisory as an action level, guidance, or enforceable standard. Some states have also adopted health action levels, guidance, or standards that are more or less protective than USEPA's health advisory. A summary of various health-based values established by states and other countries are available for download (in an Excel spreadsheet) on ITRC's website.

A summary of select state and federal values (as of October 2, 2020) is shown in the table on the next page.

Select PFAS Standards and Guidance Values in the U.S. (in ppt)											
Specific PFAS	NHDES MCLs	NJDEP MCLs	VT DEP MCL	MI DHHS MCL	MA DEP MCL	NY DOH MCLs	MN DOH Guid.	CA Response Level	CA Notif. Level	USEPA LHA	CT DPH Advisory
PFOA	12	13	20* combined	8	20* combined	10	35	10	5.1	70* combined	70* combined
PFOS	15	14	*	16	*	10	15	40	6.5	*	*
PFHxS	18		*	51	*		47				*
PFNA	11	13	*	6	*						*
PFHpA			*		*						*
PFDA					*						
GenX				370							
PFBS				420			2000				
PFBA							7000				
PFHxA				400,000							
All units are in part-per-trillion (ppt)											

MCL=Maximum Contaminant Level; LHA=Lifetime Health Advisory

Topic 8: USEPA's Ongoing Work to Develop New Analytical Methods

EPA is currently developing several new PFAS analytical methods. These methods include:

- SW-846 Method 8327—Direct Injection – Non-Drinking Water Sample Method that can be used for surface water, groundwater, and wastewater. This method will include 24 analytes, including all the Method 537.1 analytes, but will have reporting limits several times higher than Method 537, 537.1 or 533. Analytical methods that utilize direct injection methods instead of extraction methods require less sample preparation time. The method is intended to be a relatively inexpensive screening method.
- CWA 1600 Method—Isotope Dilution – This method will likely be more robust for complex matrices in liquid or solid forms (e.g., wastewater influents, leachates, soils, sediments, and biosolids) and account for matrix effects (e.g., sorption) through isotopically-marked standard recoveries.
- Total Organofluorine Analysis using Combustion Ion Chromatography (TOF by CIC) - This method can be used on aqueous matrices and blood samples. The method will likely have relatively high reporting limits due to the need to remove background inorganic fluorine from the sample to ensure only organic fluorine is reported. Analyzing total organofluorine will provide a measurement of the total amount of man-made fluorochemicals that are in drinking water. This will help provide a holistic snapshot of the occurrence of these compounds and critical information for water systems and policy makers on steps that need to be taken to protect our nation's water supply. This information will also ensure the adequacy of currently available PFAS analytical methods in identifying exposure to these compounds.

EPA is developing additional methods for serum, air, marine waters, and non-targeted analyses.

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