Per- and Polyfluoroalkyl Substance Testing in Clinical Specimens

Guidance for Laboratories

Per- and polyfluoroalkyl substances (PFAS) persist in the environment, with some of these compounds retained and slowly eliminated in humans. With increased detection of PFAS in drinking water, food packaging materials and consumer products, there is a concurrent interest in testing clinical specimens for PFAS. This document outlines best practices for sampling and testing applicable to public health, clinical and commercial diagnostic, and research laboratories seeking to measure PFAS in human biological samples.

For the purposes of this document, types of clinical PFAS testing include and are defined as:

- **Biomonitoring:** The measure of chemicals in biological specimens coupled with exposure information, to inform public health intervention and to identify trends and emerging concerns.
- **Clinical Screening:** The chemical analysis of biological specimens without paired exposure information, for which there are no health-based reference values.
- **Diagnostic Testing:** The analysis of biological specimens to measure chemicals for comparison to health-based reference values to inform individual medical management. Currently, there is no diagnostic testing available for PFAS.

Specimen Types

Serum is the universally accepted clinical specimen for measuring PFAS. In the United States, programs like the National Health and Nutrition Examination Survey (NHANES) have measured PFAS in serum for decades, enabling comparisons of serum measurements across investigations. The *National Report on Human Exposure to Environmental Chemicals* (National Exposure Report) presents nationally representative, cumulative biomonitoring data gathered by NHANES since 1999–2000. If serum is not available, plasma is the secondary choice of specimen.

The use of whole blood, dried blood spots, hair, breast milk and urine are not recommended for biomonitoring, clinical screening or diagnostic testing. If testing of whole blood, dried blood spots, hair or urine is conducted, data obtained should be used for research purposes only as there is not sufficient data available for comparison and results interpretation.

Figure 1. Specimen types for clinical PFAS testing*

Specimen Type	Advantages	Limitations	Recommended For:
Serum	• Primary biological distribution medium for many PFAS • Highest concentrations of most PFAS • Medium for most biomonitoring studies • Established quality assessment program • Available reference materials	Invasive collection	• Biomonitoring • Clinical screening • Research
Plasma	• Similar concentrations of PFAS compared to serum • Some biomonitoring studies done using plasma • Available reference materials	Invasive collection	• Biomonitoring • Clinical screening • Research
Whole blood	Options for venous or capillary collection	• Most PFAS bind to serum proteins, not blood cells • Concentrations are about half those in serum/plasma • Invasive collection • No quality assessment programs • No available reference materials • Not recommended for general population exposures	Research purposes in affected communities expected to have relatively high PFAS concentrations
Breast milk		• No generally accepted methodology • Concentrations closer to those in urine than in serum/plasma • No quality assessment programs • No available reference materials	Toxicokinetic research studies
Urine	Non-invasive collection	• Concentrations are much lower than in serum/plasma • No data available for comparison • Limited quality assessment options • No available reference materials	Toxicokinetic research studies
Dried blood spots		• Most PFAS bind to serum proteins, not blood cells • Concentrations about half those in serum/plasma • External contamination of cards • Small sample volume • No data available for comparison • No quality assessment programs • No available reference materials	Not recommended
Hair	Non-invasive collection	• PFAS are unlikely to be detectable • Challenging to analyze • Significant exogenous contamination • No data available for comparison • No quality assessment programs • No available reference materials	Not recommended

* Specimen types missing from this table (e.g., toenails) should be considered impracticable.

Specimen Collection and Storage

To obtain high-quality samples, contamination must be minimized when collecting, storing and managing biological specimens, as pre-analytical contamination could compromise accurate results. Diligence in preventing contamination is particularly important for specimens analyzed for PFAS due to the abundance of PFAS in the environment.

Collection

Minimizing the likelihood of specimen contamination is essential to obtain accurate results. As PFAS are pervasive in the environment, quality practices are necessary throughout the specimen collection and transport, storage and analysis processes. Sample collection supplies (blood tubes and other materials) and storage containers (cryovials) should be prescreened for PFAS to verify they are free from contamination prior to sample collection. Materials with polytetrafluoroethylene (PTFE) and polyvinylidene fluoride (PVDF) coatings should be avoided.[1] If prescreening is not possible (e.g., specimens were initially collected for a different purpose), screening blank materials ("field blank") of the same lot used to collect samples is recommended to aid in interpretation of results. Specimens may need to be collected again if pre-analytical contamination is suspected or cannot be ruled out, because it may result in inaccurate or unreliable test results.

The specimen volume required for analysis will be method dependent, but collection of a volume exceeding the minimum amount required for analysis is prudent to account for reanalysis. Refer to the laboratory's standard operating procedures (SOPs) for details on specimen rejection criteria including whether low volume samples are acceptable.

Serum samples are most frequently collected using red-topped vacutainers/tubes; however, serum separator tubes have been used successfully and may be helpful to collect samples where immediate processing is not feasible. For plasma, lavender-topped tubes with EDTA preservative are preferred over green-topped tubes with heparin preservative, but both can be used successfully. Citrate and other preservatives are not recommended for plasma. In all cases, tubes should be screened to verify they are free from PFAS contamination or interfering compounds.[1]

Storage

PFAS serum and plasma samples are typically stored frozen in polypropylene or polyethylene cryogenic vials with threaded caps at temperature of −70°C.[2] Specimens should be transferred to cryogenic vials shortly following collection. Cryogenic vials with externally threaded caps are preferred to minimize cross-contamination and prevent leakage. Internally threaded containers and flip-top microcentrifuge tubes may not provide a sufficient seal and are not recommended.

Best practices and further guidance on general specimen collection, transfer and storage procedures are provided in CDC's *Improving the Collection and Management of Human Samples Used for Measuring Environmental Chemicals and Nutrition Indicators*.[1]

Quality Management System

A quality management system (QMS) describes how laboratories perform internal audits to ensure acceptable performance and assures the integrity and traceability of laboratory results. The QMS guides a laboratory in implementing an overall quality policy that encompasses quality assurance (QA; plans that guide laboratory activities) and quality control (QC; measures to ensure compliance); both are required elements of the overarching QMS.[3]

QA incorporates those planned and systematic laboratory activities that guarantee the accuracy and defensibility of testing results. Quality manuals, SOPs and documentation are essential components of a QA program. "Quality manual" refers to the master document of the laboratory quality policy and serves as the primary resource for laboratory information. Laboratory staff are responsible for ensuring adherence to the laboratory quality manual, QA plan and SOPs. Other supplementary records (e.g., instrument logbooks, reporting forms) are also critical components in a QA program.[3]

QC samples—known concentrations of PFAS in matrix—are run concurrently with test samples to monitor the efficiency of extraction and instrument performance. Laboratories must establish acceptable criteria for QC samples and investigate potential causes for non-conformance and implement corrective actions when concentrations in QC samples are out of range.

Laboratories must include processes that can adequately evaluate data quality in the pre-analytical (e.g., obtaining patient information, collecting biospecimens), analytical and post-analytical phases (e.g., data analysis, report generation).

Laboratories testing clinical specimens for PFAS should investigate which certifications and accreditations are indicated for the intended type of testing, particularly for laboratories who plan to report results back to individuals. Generally, Clinical Laboratory Improvement Amendments (CLIA) program or comparable certification is required to perform this testing.

External quality assessment programs—ideally, certified proficiency testing programs—are performed to evaluate the laboratory's ability to accurately identify and measure analytes of interest.[3] Laboratories performing this testing must be able to demonstrate proficiency through regular successful participation in an external quality assessment program. The Centre de Toxicologie du Québec's AMAP[4] program (includes all analytes recommended in this guidance) and the German External Quality Assessment Scheme[5] are two proficiency testing programs that offer coverage of at least some PFAS recommended in this guidance.

If a laboratory is interested in testing additional PFAS not covered by the above programs, the laboratory should consider internally blinded samples to evaluate performance. Information on harmonization of laboratory measurements is available in the Association of Public Health Laboratories' *National Biomonitoring Network Strategy for Harmonization of Laboratory Measurements*.

Analytical Considerations

Recommended Analytes

The specific PFAS tested by a laboratory is influenced by the purpose of testing and laboratory capability. Laboratories analyzing samples for biomonitoring may have a broader analyte panel while laboratories performing clinical screening may utilize a limited analyte panel. Not all PFAS included in the list may be detected in clinical specimens, and the detection frequency may vary depending on geographic area, exposure scenario, exposure time and more (e.g., legacy PFAS like PFOA and PFOS may be detected at lower concentrations in children than adults from the general population).

Figure 2: Recommended Minimum and Advanced PFAS Testing Panels

	Minimum	Advanced
Applies to:	All laboratories	Laboratories with advanced analytical capability and an interest in more comprehensive testing
Recommended Testing Panel	• **PFHxS** (Perfluorohexane sulfonic acid) • **PFOS** (Perfluorooctane sulfonic acid) • **PFOA** (Perfluorooctanoic acid) • **PFNA** (Perfluorononanoic acid)	• **PFHxS** (Perfluorohexane sulfonic acid) • **PFOS** (Perfluorooctane sulfonic acid) • **PFOA** (Perfluorooctanoic acid) • **PFNA** (Perfluorononanoic acid) • **PFDA** (Perfluorodecanoic acid) • **PFUnDA** (Perfluoroundecanoic acid) • **MeFOSAA** (N-methyl-perfluorooctane sulfonamidoacetic acid)
Inclusion Rational	These are the most prevalent PFAS reported in the National Exposure Report for the US population, which have been included in many biomonitoring studies both in the US and abroad and have the most information available in existing scientific literature.	These PFAS are included in the National Exposure Report and the 2022 Guidance on PFAS Exposure, Testing, and Clinical Follow-Up and have the most recent publicly available data available.

Analytical Parameters

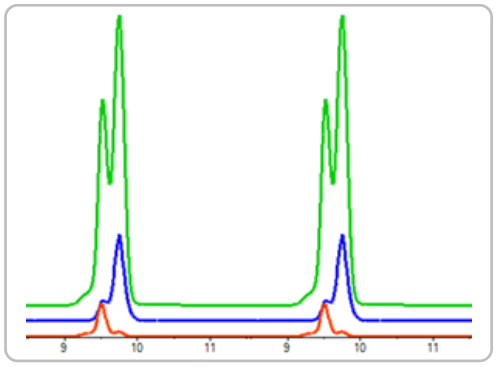
For identification and quantitative analysis of clinical specimens for individual PFAS, targeted testing via liquid chromatography-tandem mass spectrometry (LC-MS/MS), utilizing a triple quadruple mass spectrometer (QQQ) is strongly recommended because of the enhanced sensitivity compared to other MS/MS techniques. Isotope dilution analysis using matching isotopically labeled internal standards of target analytes is recommended.

Currently, semiquantitative and nontargeted analysis using high resolution mass spectrometry (HRMS) are not recommended for testing of clinical specimens due to the high potential for misidentification of PFAS and uncertainty in confidence and concentration. Results obtained from nontargeted testing are not appropriate for application to risk or health-based decision making. Nontargeted analysis is a developmental technique with evolving utility in the detection of PFAS and is currently best suited for screening or research purposes.

Measurement Strategies

PFAS concentrations may be quantified as linear and branched isomers or as total measurement. Appropriate measurement may differ depending on the purpose of testing, but measurement of total PFAS comprising both linear and branched PFAS will yield the most valuable data. For some PFAS like PFOA, detection of branched isomers is unlikely. To ensure high accuracy and quality of results, standards should be only purchased from reputable suppliers.

Figure 3. Example chromatogram of overlaid MS/MS transitions showing branched PFOS in serum sample



Contamination Prevention and Control

Laboratories performing clinical PFAS testing should maintain a dedicated instrument exclusively for analysis of human clinical specimens for PFAS. Additional sample types like food, water or other environmental samples should not be analyzed using the same instrument as clinical PFAS samples. Potential sources of PFAS contamination in the laboratory include solvent lines, HPLC components, aluminum foil and solvents. Tubing, equipment or other instrument parts manufactured with PFAS should be avoided and laboratories should ideally maintain a PFAS-free instrument. Instrument manufacturers now provide technical notes and sell "kits" for instruments utilized for PFAS testing. Laboratory equipment and supplies should be examined for PFAS prior to testing clinical specimens.

Results Interpretation and Return

Laboratories performing testing for PFAS in clinical specimens must maintain CLIA or comparable certification, which requires some level of interpretation on their report. Best practices for returning results to biomonitoring participants are described in APHL's *Guidance for Laboratory Biomonitoring Programs* and National Academies of Sciences, Engineering, and Medicine's *Returning Individual Research Results to Participants: Guidance for a New Research Paradigm*.

Additional resources for results interpretation and application are available from the following sources:

- National Academies: Guidance on PFAS Exposure, Testing and Clinical Follow-up
- ATSDR: PFAS Information for Clinicians
- ATSDR: Exposure Assessment Results from 10 communities with contaminated water

References

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Association of Public Health Laboratories

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