SERDP

EXECUTIVE SUMMARY

Improving Access and Utility of Analytical Data for the Confident Discovery and Identification of PFAS in Environmental Matrices

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SERDP Project ER20-1056

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ACRONYMS AND ABBREVIATIONS

AFFF	aqueous film-forming foams
DIMSpec	Database Infrastructure for Mass Spectrometry
DIMSpec-QC	DIMSpec quality control
ECF	electrochemical fluorination
FAIR	findable, accessible, interoperable, and reusable [principles]
JSON	JavaScript object notation
LC-HRMS	liquid chromatography – high resolution mass spectrometry
MSMatch	Mass Spectral Match [for Non-Targeted Analysis]
NIST	National Institute of Standards and Technology
NTA	non-targeted analysis
NTA-MRT	Non-Targeted Analysis Method Reporting Tool
OECD	Organization for Economic Co-operation and Development
PFAS	per- and polyfluoroalkyl substances
PFAS-NTAILS	PFAS non-targeted analysis interlaboratory study
QA/QC	quality assurance/quality control
QAP	quality assurance program
USEPA	United States Environmental Protection Agency

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Finally, the project team would like to recognize the entire National Institute of Standards and Technology (NIST) PFAS Program research team for their work in support of this project, including Alix Rodowa, Amy Cuthbertson, Niksa Blonder, Carolyn Burdette, Catherine Rimmer, John Kucklick, Nathan Mahynski, Carlos Gonzalez, and Katherine Peter (former NIST postdoctoral fellow).

1.0 INTRODUCTION

1.1 PRESENCE OF PFAS IN THE ENVIRONMENT FROM UNKNOWN SOURCES

Per- and polyfluoroalkyl substances (PFAS) are a large class of anthropogenic chemicals with unique properties (Kissa, 2001); a subset of PFAS are known to be persistent, bioaccumulative, and/or toxic to humans and the environment (see Bartell & Vieira, 2021; Evich et al., 2022; and Fenton et al., 2021 and sources therein). Depending on the definition, there can be over 10,000 chemicals that are considered PFAS (Buck et al., 2011; Gaines et al., 2023; Organization for Economic Co-operation and Development (OECD), 2021). Currently, there are regulations for six PFAS in drinking water (40 CFR § 141 (2024), 40 CFR § 142 (2024) and two PFAS are designated as hazardous substances through the Comprehensive Environmental Response, Compensation, and Liability Act (40 CFR § 302 (2024)).

Over a decade of research has demonstrated the existence of PFAS beyond perfluoroalkyl carboxylic acids and perfluoroalkyl sulfonic acids in environmental materials due to a range of sources, including the release of aqueous film-forming foams used for fighting liquid fuel-based fires. (Backe et al., 2013; Barzen-Hanson et al., 2017). While the other PFAS may not be directly regulated, there is evidence demonstrating that many of these PFAS can transform into PFAS of concern in the environment. This brought about the rise of the term "PFAS Precursors" to indicate chemicals that transform into perfluoroalkyl acids through abiotic and biological processes (Ruyle et al., 2023).

1.2 ROLE OF NON-TARGETED ANALYSIS FOR THE IDENTIFICATION OF PFAS

Non-targeted analysis (NTA) is a category of techniques that intend to identify chemicals in a sample with limited-to-no *a priori* knowledge regarding the chemical composition and quantities within the sample. This includes the identification of previously unknown chemicals in environmental samples where no chemical standards exist for performing conventional targeted, quantitative analysis. As there can be thousands of PFAS that could exist in the environment, and chemical standards are limited to less than 100 PFAS, the use of NTA is well-suited for the detection and identification of PFAS in a wide range of samples. In fact, NTA techniques have been routinely applied to materials containing PFAS and PFAS-impacted environmental samples over the past decade, resulting in the identification of previously unknown PFAS in the environment (Barzen-Hanson et al., 2017; Place & Field, 2012).

Previous studies have used NTA to identify PFAS by elucidating the chemical structure through interpretation of analytical data and other supporting information (such as patents). While this approach can be used to identify truly novel PFAS, it is time-consuming, and the confidence of the identification can be limited. Comparison of measured fragmentation mass spectra with reference mass spectra of known compounds provides one of the highest levels of confidence in compound identification. But this approach is limited to the availability of reference mass spectra. Conventionally, libraries containing reference mass spectra are developed based on generated mass spectra using chemical standards, but there are not many chemical standards for the number of possible PFAS.

1.3 IMPROVING DATA ACCESS TO ENABLE NON-TARGETED ANALYSIS FOR PFAS

The mass spectral data produced from individual laboratories analytical instrumentation are generated in a vendor-specific proprietary data format. This can include lists of chemical suspects and reference mass spectra. This condition limits the ability of individual laboratories to share their data with other laboratories, especially those that have different vendor instruments. It is important to understand the current needs and predict future needs for data use, which includes the systematic collection of metadata that is relevant for current data use or could be relevant for future data investigations. The database developed through the first objective of this study needed to be interoperable and accessible to provide impactful data and tools that improve NTA for PFAS identification.

2.0 OBJECTIVES

The primary objective of this research was to enable analytical laboratories to better identify novel PFAS by improving access to high-quality reference mass spectra and providing a comprehensive quality assurance program (QAP) for PFAS identification via NTA. These objectives were accomplished through two tasks:

Task 1: Develop a common database structure for the detection and identification of PFAS.

Task 2: Establish a QAP aimed at developing and qualifying analytical laboratories for the detection and identification of PFAS in environmental matrices.

3.0 TECHNICAL APPROACH

3.1 INTERNAL GENERATION OF REFERENCE MASS SPECTRA

Internal generation of reference mass spectra for PFAS was performed using two different sources of materials: commercially available standard mixtures and synthesized single component solutions (acquired through the USEPA). Standard mixture solutions were analyzed directly without additional sample preparation. Single component standard solutions were combined into a single solution by 1:50 (by volume) dilution in methanol. Additional solutions were acquired as part of a material transfer agreement with the USEPA, entitled “Per- and Polyfluoroalkyl Substance (PFAS) Standards”. The set of PFAS solutions will be referred to as the USEPA PFAS150 standard set in this report. The USEPA PFAS150 standard set contained 141 individual PFAS in solution that were generated by a chemical manufacturer; many of the PFAS were not commercially available otherwise.

Reference mass spectra for these samples were generated using a standardized liquid chromatography – high resolution mass spectrometry (LC-HRMS) workflow and quality control procedures that enabled high quality and reproducible mass spectra. Generally, the LC-HRMS method included a reversed phase separation using specific mobile phases for the ionization polarity (0.1 % formic acid in water and methanol for positive ionization and 10 mM ammonium acetate in water and methanol for negative ionization). Mass spectrometric analysis was performed using a Thermo Fisher Scientific Q-Exactive hybrid mass spectrometer (Waltham, MA) and full scan MS1/data-dependent MS2 experiments for all compounds; this method allowed for the production of high-quality mass spectra with minimal interferences from co-eluting compounds.

For quality control, a novel approach was developed using standard solutions containing known compounds that were analyzed within the above-described workflow at least twice within an analytical sequence. Quality control parameters, including mass accuracy, retention time stability, chromatographic peak parameters, and mass spectrum reproducibility, were analyzed using in-house developed R scripts to verify that the within-sequence accuracy and precision were within tolerable limits.

All reference mass spectra generated through the above-described internal procedures were inspected manually, annotated using the Non-Targeted Analysis Method Reporting Tool (NTA-MRT), and stored until they could be incorporated into the developed database. The NTA-MRT is available at https://github.com/usnistgov/NISTPFAS.

3.2 EXTERNAL ACQUISITION OF REFERENCE MASS SPECTRA

Additional reference mass spectra were generated using data from published peer-reviewed literature. Specifically, the Dr. Jennifer Field laboratory at Oregon State University provided the data from Barzen-Hanson et al. (2017). Only data produced from aqueous film-forming foams (AFFF) and technical solutions were used to generate the reference mass spectra.

3.3 TEST MATERIAL DEVELOPMENT FOR A NON-TARGETED ANALYSIS INTERLABORATORY STUDY

For the administration of the PFAS non-targeted analysis interlaboratory study (PFAS-NTAILS), a set of three test solutions were created that contained both known and unknown PFAS. The solutions include:

- **Sample A**, which was a solution consisting of a methanolic dilution of multiple analytical standards of PFAS (also in methanol). The nominal concentration was 0.1 µg/g for all PFAS components.
- **Sample B**, which was a solution consisting of a methanolic dilution of two AFFF commercial solutions. One of the AFFF solutions was an electrochemical fluorination (ECF) based product, and the second solution was a fluorotelomerization based product.
- **Sample C**, which was a methanolic extract of an AFFF-impacted soil amended with an analytical standard of a single PFAS in methanol.

All solutions were ampuled in 2023 and placed into boxes for storage. Boxes of ampoules were stored at 4 °C until safety labeling and shipment.

4.0 RESULTS AND DISCUSSION

4.1 TASK 1: DEVELOPMENT OF A COMMON DATABASE STRUCTURE FOR THE DETECTION AND IDENTIFICATION OF PFAS

4.1.1 Establishment of the NIST PFAS Suspect List of Possible Per- and Polyfluoroalkyl Substances

To address the need for a well-curated list of PFAS structures that could be detected using NTA workflows, NIST developed a suspect list of structures of the possible PFAS, which included empirically observed and *in silico* predicted structures. NIST developed an automated protocol that required specified input values to address this data gap and provide a more robust, and easily updated, suspect list for PFAS, called the "NIST List of Possible Per- and Polyfluorinated Alkyl Substances (PFAS)" or the "NIST PFAS Suspect List" for short. The suspect list is provided to the community via the NIST public data repository (B. Place, 2021) that is maintained by the NIST PFAS Program so that it may be easily available, updateable, and version controlled.

Aggregation of the initial suspect list used two primary sources of data: the OECD PFAS list from the USEPA CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard/) and the PFAS XIC (extracted ion chromatogram) List provided by Dr. Christopher Higgins (Colorado School of Mines; ORCID: 0000-0001-6220-8673; chiggins@mines.edu). Since the initial release of the PFAS Suspect List, several additional PFAS structures have been contributed by individual laboratories. As of release of this report, the current PFAS Suspect List (v1.7, https://data.nist.gov/od/id/mds2-2387) contains 4,967 individual PFAS structures.

4.1.2 Development of a Database Infrastructure for Mass Spectrometry (DIMSpec)

A database toolkit was developed to provide an infrastructure for the management and use of mass spectrometry data and associated metadata. This toolkit was named the Database Infrastructure for Mass Spectrometry (DIMSpec), with this project's specific application for PFAS, although the database will be solely referred to as DIMSpec for the rest of this report. In addition, as part of a NIST-wide effort to make data more compliant with the FAIR (Findable, Accessible, Interoperable, and Reusable) principles (Wilkinson et al., 2016), the database and affiliated tools were designed using open-source formats that can be easily shared and reused by laboratories within and outside of NIST. The information provided in this report includes an overview of guidance for the setup, population, and use of DIMSpec databases and its affiliated toolkit. Complete instructions and more detail are available in the User Guide (https://pages.nist.gov/dimspec/docs), which will be updated as the project continues.

The database schema for DIMSpec databases is described in detail in the User Guide. The utility of DIMSpec for NTA was greatly enhanced by providing controlled vocabulary in terms of normalization tables, and all chemical entities were cross-linked with additional validated names (where available) such as database identifiers from CompTox and PubChem, common acronyms, and machine interpretable structure notation in the form of International Chemical Identifier and Simplified Molecular Input Line Entry System to prevent ambiguity in chemical identifier (Place & Ragland, 2022).

Additional information regarding the design and infrastructure of the database is provided in more detail in Ragland & Place 2024 and in the User Guide (https://pages.nist.gov/dimspec/docs).

A cartoon diagramming the overall conceptual structure of the DIMSpec project is provided (**Figure ES-1**), as is the full entity relationship diagram of the underlying database schema in the supporting documentation.

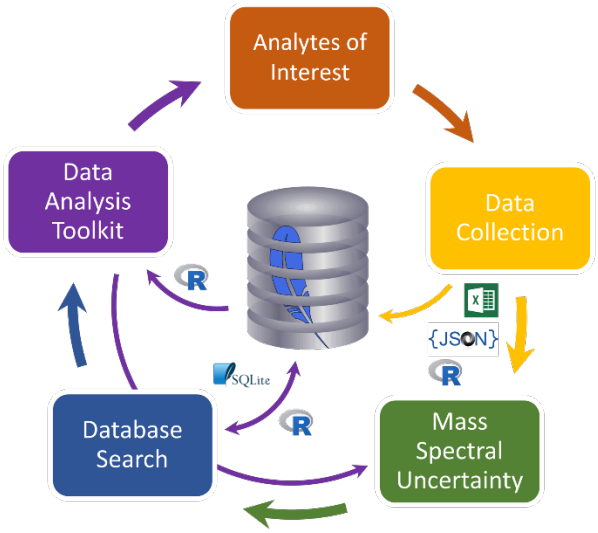

Figure ES-1. Conceptual Diagram of the DIMSpec Toolkit.

4.1.3 Non-Targeted Analysis Method Reporting Tool (NTA-MRT)

To assist with annotation of chemical identity and observed fragmentation patterns, a macro-enabled Microsoft Excel workbook was developed that "allows for the controlled ontology of method data reporting and the export of the data into a single concise, human-readable file, written in a standard JavaScript Object Notation (JSON)." To annotate the data intended for inclusion in a DIMSpec database, users fill out this workbook by annotating features of interest and associated fragmentation identities. This tool is freely available via GitHub and instructions for completing it are contained within the workbook itself (https://github.com/usnistgov/NISTPFAS/tree/main/methodreportingtool).

4.1.4 Quality Assessment of Imported Data (DIMSpec-QC)

A DIMSpec mass spectral database incorporates empirical mass spectral data from analytical standards and complex mixtures with relevant analytical method metadata and mass spectral annotation. Algorithms were developed in R to validate the quality of new experimental data for import into a DIMSpec database, specifically to ensure that annotations "made sense" when applied to the spectra collected. For ease of use, the DIMSpec Quality Control (DIMSpec-QC) application was developed that incorporates the R functions into a web application powered by the Shiny package (Chang et al., 2024). Scripts for automated setup are included, and DIMSpec-QC installs along with the project. The primary purpose of the DIMSpec-QC application is to apply quality control checks to NTA-MRT-generated files which may then be imported using functions from the toolkit.

4.1.5 Development of Data Analysis Tools for DIMSpec (MSMatch)

The Mass Spectral Match for Non-Targeted Analysis (MSMatch) application was built to accelerate non-targeted analysis projects by searching experiment result data in mzML format for matches against a curated mass spectral library of compounds and annotated fragments. MSMatch is a web application built using the Shiny package in R and installs alongside DIMSpec. Every effort has been made to make MSMatch as intuitive for users as possible. The data analysis tools include two specific approaches to identifying PFAS: the compound match tool and the fragment match tool.

4.1.5.1 Compound match

The compound matching function of MSMatch examines experimental mass spectra (unknowns) against mass spectra known to the attached DIMSpec database. The compound matching algorithm can be used to search against all mass spectra or against mass spectra with precursor *m/z* values within an acceptable range. An evaluation of match score uncertainty is also provided. The calculation of mass spectral uncertainty and estimation of the distribution of the match scores is described in B.J. Place 2021.

4.1.5.2 Fragment match

In addition to the compound match function, the fragment matching function provides information regarding the individual fragments of the input mass spectra (unknowns). This can be used in combination with the compound match function; in addition, this application can be used when there are no good compound matches to provide substructure information. Fragments measured within the feature of interest will be matched against database fragments with known annotations.

Data produced internally and acquired externally were incorporated into the database. As of this report, 351 consensus reference mass spectra representing 132 individual PFAS are included. This list is accurate only as of this report, as community members are beginning to submit spectra for consideration; DIMSpec databases are intended to grow over time to provide increased value.

4.2 TASK 2: ESTABLISHMENT OF A QAP AIMED AT DEVELOPING AND QUALIFYING ANALYTICAL LABORATORIES FOR THE DETECTION AND IDENTIFICATION OF PFAS IN ENVIRONMENTAL MATRICES.

4.2.1 Development of Education and Training Tools for DIMSpec

With the public release of DIMSpec and the publication of Ragland & Place 2024, NIST researchers generated a document to provide users with an understanding on the installation and development of the DIMSpec infrastructure and the use of the associated applications.

The first form of education and training was presented as a detailed user guide, which is publicly available at https://pages.nist.gov/dimspec/docs/index.html. This user guide includes a general introduction and installation instructions for DIMSpec applications. In addition, detailed information for the associated applications (Table Viewer, DIMSpec-QC, and MSMatch) are provided as part of the user guide. In addition to this user guide, a set of quick start guides were developed for DIMSpec and the associated applications to enable more efficient use of the tools.

4.2.1.1 Training videos

While the documentation was provided for reference, users identified the value in having additional resources for training on the use of DIMSpec and its associated applications. There were five videos (called "episodes") that were created to communicate the installation and use of DIMSpec and the associated applications. The videos are located on the NIST site at: https://www.nist.gov/programs-projects/and-polyfluoroalkyl-substances-pfas/research/reference-data-and-tools/dimspec. The videos were made available prior to the initiation of the interlaboratory study, to enable the participating laboratories to use the guides and videos to operate DIMSpec and its associated applications for their NTA workflows.

4.2.2 Results of PFAS NTAILS

Recruitment of participants (up to 50 laboratories) for the interlaboratory study started on February 1, 2024, and ran until March 1, 2024. The three test samples (described previously) were shipped to 34 participating laboratories. Of the participating laboratories, 27 submitted NTA results. Of the laboratories that submitted data, 11 laboratories were from academic institutions, three laboratories were analytical instrument vendors, six laboratories were commercial/contract analytical laboratories, and seven were from government organizations (U.S. Federal, State, and non-U.S.).

For all samples, summary tables included the reporting rates of the top twenty compounds, which were defined as the twenty most frequently reported compounds (highest reporting rates) in each respective sample.

4.2.2.1 Sample A

Sample A was a solution of multiple analytical standards for PFAS. Nearly all PFAS that were intentionally added to Sample A were reported by greater than 70 % of participating laboratories, except for 5:3 fluorotelomer betaine (NISTPFAS003794, 26 % reporting rate). Due to its quaternary amine functional group, 5:3 fluorotelomer betaine can only be detected in positive ionization mode and its detection may have been limited to those laboratories that used positive ionization in their methods. The reporting rate of the top twenty compounds ranged from 96 % (26 out of 27 laboratories) to 19 % (5 out of 27 laboratories). No individual PFAS in Sample A was detected by all the participating laboratories.

4.2.2.2 Sample B

Sample B was a mixture of two different AFFF commercial formulations diluted in methanol. It contained PFAS synthesized using ECF and telomerization processes, therefore there are multiple classes of PFAS that could be present. Of the top twenty reported PFAS, ten were identified at a Level 1a confidence by at least one laboratory (the confidence levels are described by Charbonnet et al., 2022). No individual PFAS in Sample B was detected by all the participating laboratories.

4.2.2.3 Sample C

Sample C was a methanolic extract of an AFFF-impacted soil, with a single added compound. Of the top twenty identified PFAS, seventeen were reported at a Level 1a confidence by at least one laboratory. No compound in Sample C was reported by all the participating laboratories. In general, there were more compounds reported by multiple laboratories than in Samples A and B.

Further targeted analysis of this material would be needed to verify the majority of the PFAS identified in this sample.

4.2.2.4 Overall Results

There was a wide distribution in the number of PFAS identities reported by individual laboratories (**Figure ES-2**). Notably, for all three samples, no single PFAS was reported by every lab (100 % reporting rate). This result could be due to interlaboratory differences in detecting PFAS or in reporting their identities. Generally, most laboratories reported the presence of those PFAS known to NIST to be present in the samples. All known PFAS were reported at Level 1a confidence by at least one laboratory.

Sample A had the broadest range of reporting rates within the top twenty most commonly identified PFAS (96 % to 19 %), while Sample C had the smallest (96 % to 78 %). This result could be due to the increasing complexity from Sample A to Sample B to Sample C; where the samples with more PFAS present at detectable concentrations resulted in a greater number of frequently reported PFAS.

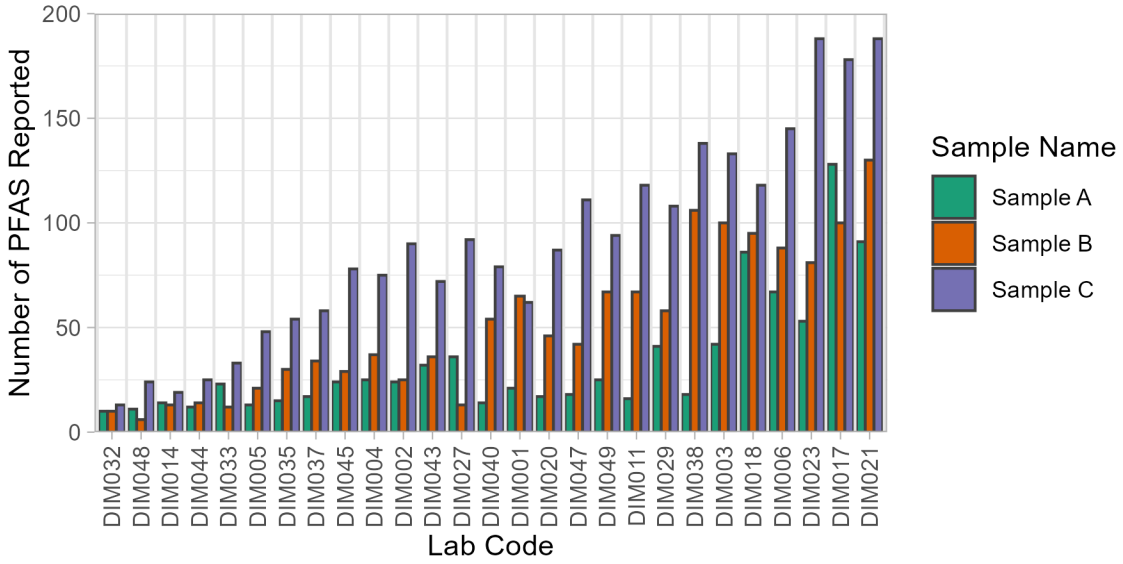

Figure ES-2. Total Number of PFAS Identified (y-axis) for Each Participating Laboratory (x-axis) by the Individual Samples.

Labs are ordered by increasing mean number of PFAS identified.

Participating laboratories either used negative ionization only or both positive and negative ionization for the analysis of the samples. The distribution of PFAS detected in positive ionization mode, negative ionization mode, and both ionization modes are shown in **Figure ES-3**. For all samples, the majority of identified PFAS were detected using negative ionization, although many PFAS were detected using positive ionization only. As less than half of the participating laboratories (44 %) used both positive and negative ionization modes, the compounds detectable only by positive ionization (such as 5:3 fluorotelomer betaine) would have been missed by majority of the participating laboratories.

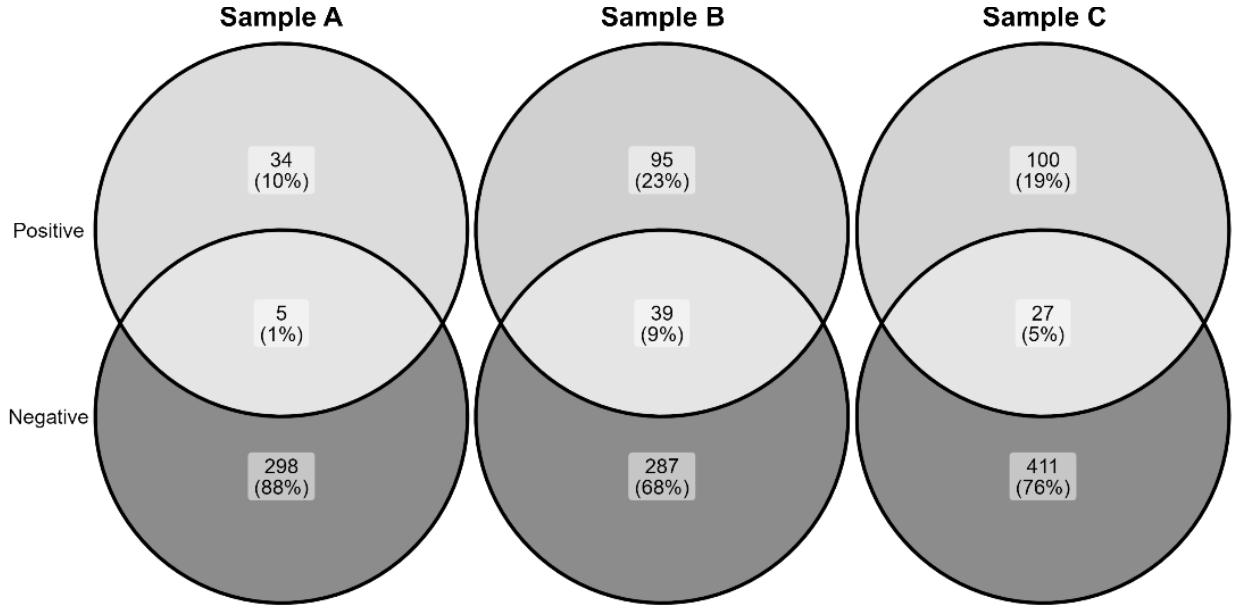

Figure ES-3. Venn Diagram Showing the Number of Individual PFAS that Were Reported in Each Sample and the Respective Ionization Polarities with Which They Were Detected.

Top: PFAS identified by positive polarity only; Bottom: PFAS identified by negative polarity only; Middle: PFAS identified by both positive and negative polarity.

5.0 IMPLICATIONS FOR FUTURE RESEARCH AND BENEFITS

5.1 DEVELOPMENT OF A COMMON DATABASE STRUCTURE FOR THE DETECTION AND IDENTIFICATION OF PFAS

The presented work resulted in the successful development of an accessible and interoperable database for the access to non-targeted analytical data for the identification of PFAS. As of 15 October 2024, the NIST Suspect List of Possible PFAS has been downloaded 1,373 times by 1,154 unique users and the DIMSpec database has been downloaded 441 times by 430 unique users. These statistics are gathered from the NIST Public Data Repository and do not include downloads directly from Github which does not track such metrics; therefore, it is expected that these values underestimate the total number of downloads and unique users. This work included frequent interactions between NIST and multiple mass spectrometer vendors, which shows promise for the continued interoperability of this database.

5.2 ESTABLISHMENT OF A QAP AIMED AT DEVELOPING AND QUALIFYING ANALYTICAL LABORATORIES FOR THE DETECTION AND IDENTIFICATION OF PFAS IN ENVIRONMENTAL MATRICES.

There were 27 laboratories that were able to participate in this interlaboratory study and provide results. Generally, most laboratories were able to identify PFAS that were present in the samples (as confirmed by internal, targeted measurements). Some laboratories reported a larger number of PFAS identities in all samples than most laboratories, including a solution containing a limited

number of spiked compounds. While the additional identities could not be confirmed or denied, it was unlikely that all these compounds were truly present in the sample. The disparity of the number of PFAS identities reported between laboratories may indicate quality assurance/quality control (QA/QC) issues, such as background contamination or a poor understanding of accuracy of individual laboratories' NTA methods. Currently, there are no broadly accepted QA/QC protocols for NTA methods, although community working groups, such as BP4NTA (Place BJ, 2021), are working to identify best practices for QA/QC of NTA methods.

Future work should examine the possibility of developing metrics for estimating true positive rates in unknown samples and the use of blank controls to account for within-laboratory contamination. The intention of this study was to evaluate the NTA workflow; therefore samples were provided as solvent extracts of environmentally relevant matrices. Future work could evaluate laboratories' abilities to extract environmental materials and identify PFAS in the extracts.

5.3 IMPLICATIONS FOR FUTURE RESEARCH AND IMPLEMENTATION

For the foreseeable future, DIMSpec, the infrastructure itself, and the PFAS database, will continue to be freely available on the NIST Public Data Repository and Github. Future updates to the database, including additions of mass spectra, will require continued support of the database, which could occur through internal or external (non-NIST) administration and maintenance.

There could be new applications of DIMSpec to answer additional research questions, including forensic source attribution of PFAS pollution or novel non-PFAS chemicals of concern in environmental matrices. The infrastructure was designed to be flexible and enable a wide variety of analytical and sample information to address new research questions and measurement challenges. The use of DIMSpec demonstrated the value of open and free data to support government, academia, and industry laboratories and should encourage other database developers to adopt similar approaches for data accessibility.

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