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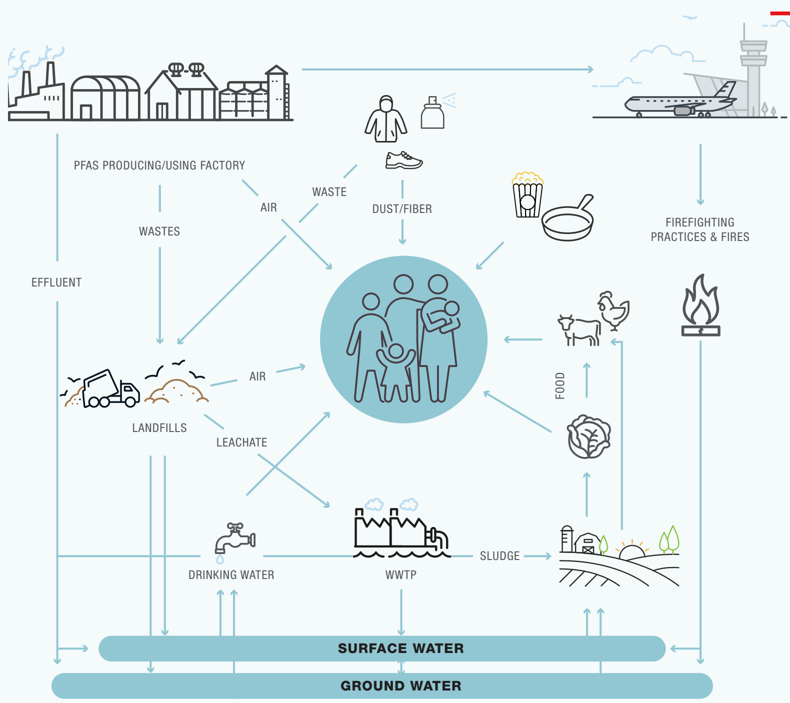
Comprehensive PFAS testing: Technologies you need to meet current and future regulatory standards

Known as 'forever chemicals,' per- and poly-fluoroalkyl substances (PFAS) are a large family of synthetic compounds used in a variety of consumer products and manufacturing processes. Due to their strong and stable carbon-fluorine bonds, these substances don't break down and continue to bioaccumulate. Consequently, they remain the most persistent environmental contaminants seen today.

Consumers are exposed to PFAS through many sources, including non-stick cookware, kitchenware, water-repellant clothing, stain-resistant fabrics, food packaging, microwave popcorn bags, and other cleaning and personal care products. PFAS also find their way into the environment, cycling through water, soil, and air, through industrial processes such as commercial manufacturing, firefighting, and waste disposal. Due to their ubiquitous nature, the Centers for Disease Control (CDC) have estimated that 99% of the population now carry PFAS in their blood. Studies have also linked PFAS to health issues such as hormonal disruption, increased cholesterol, and cancer risks.

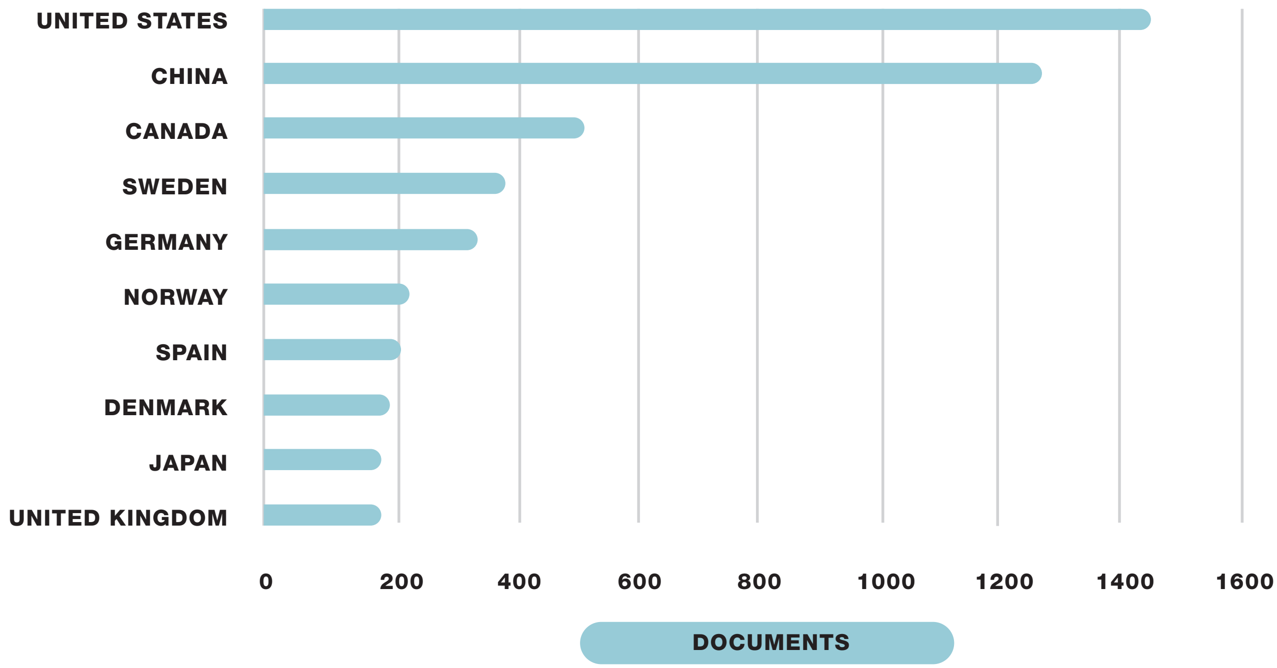


PFAS Environmental Cycle and Human Exposure

PFAS chemicals cycle through the environment daily in air, water, soil and sediments. Through this cycle, they accumulate in fish, wildlife and humans. Most people are exposed to PFAS from drinking water and eating food that contain these chemicals. According to the CDC, and the World Health Organization exposure to PFAS can increase cholesterol and cancer risks, as well as interfere with hormones and thyroid performance.

Over 5,000 variants of PFAS are estimated to be circulating in the environment today, raising public health concerns and laboratory testing challenges. In Europe, health conditions related to PFAS exposure cost a whopping €50 billion. To curb these pollutants, regulatory bodies have imposed advisory limits on commonly observed PFAS compounds. Additionally, as PFAS chemicals are so pervasive, taxpayers and companies across the world are expected to shell out billions of dollars towards mandatory PFAS testing and clean-up. In the US alone, the Department of Defense spent $1.1 billion on PFAS clean-up in 2020 and is likely to double that expenditure in 2021. Meanwhile, from a research perspective, funding towards investigating PFAS health hazards and designing alternatives is gradually increasing, and consequently, so is the scientific output related to PFAS.

Investment and focus in scientific research to understand alternative chemistries and reduce public health impact continues to grow annually and across the world.



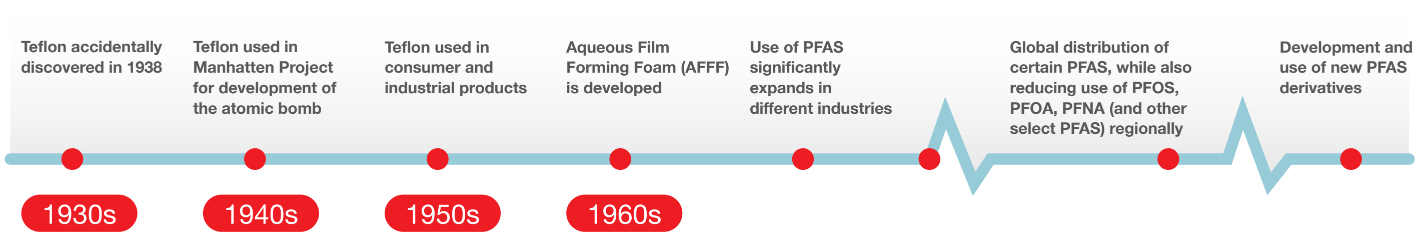
Total number of documents published by top 10 countries concerning PFAS from 2000 to 2020 (data extracted from Scopus; October 2020).

Now more than ever, with added pressure from governments to reduce human health and environmental concerns, analytical laboratories analyzing water and soil samples for traces of PFAS are required to have the necessary expertise and capabilities to comply with regulatory requirements.

Here, we provide an overview of the essential technologies and key considerations for accredited PFAS testing laboratories and researchers as they navigate local and regional regulations and growing demands for investigative testing related to site cleanups.

Keeping up with the ever-evolving PFAS regulations

Although PFAS were manufactured and used in a variety of consumer products for over 50 years, the awareness of their impact on the environment and public health has only been growing in recent times. As the scientific community's understanding of PFAS increases, regulatory bodies around the globe often update testing requirements to protect human health. This makes PFAS testing a fast-paced, dynamic field, where laboratories need to regularly keep up with changing regulations and the discovery of new contaminants.



Estimated number of fluorinated organic compounds produced globally 1950-current.

To ensure public safety, regulatory authorities continuously assess safe levels of compounds and publish analytical methods to uphold accuracy and consistency across testing laboratories. Examples of methods developed by the US Environmental Protection Agency (EPA) for drinking water include 537, 537.1, and 533, while those for non-potable water are EPA 8327 and ASTM 7979. Similarly, methods for PFAS detection in sediment and soil extracts are also standardized, while air sampling methods are currently underway. Over the years, Thermo Fisher Scientific has closely collaborated with the EPA offices, participating in the development of these PFAS methods, with many of its analytical instruments considered gold standards in the industry.

Sample Type	Method	
Drinking water	EPA 537, EPA 537.1	Internal standard, 18 analytes
Drinking water	EPA 533	Isotope dilution, 25 analytes
Non-potable water (Surface waters, groundwater, wastewater)	EPA 8327	External standard, 24 analytes
	ASTM D7979	Isotope dilution, 21 analytes
Soils	EPA 8327	External standard, 24 analytes
	ASTM D79668-17a	Isotope dilution, 21 analytes

These standardized PFAS methods utilize modern analytical techniques such as solid-phase extraction, liquid chromatography (LC), and mass spectrometry (MS), to separate, identify and quantify known PFAS in samples. In developing these validated methods, several factors need to be considered: sample type, calibration range, detection limit, screening capabilities, and quantitative accuracy, among others. As such, testing laboratories will need to have sensitive analytical systems that meet these current requirements as well as demonstrate the ability to quickly pivot and adapt to potential changes in the future. Moreover, while regulations mandate quantifying known PFAS compounds, laboratories benefit from extending their investigational capabilities by incorporating technologies that can identify unknown compounds or rising areas of concern.

As a direct consequence of recent updates in PFAS regulations, a surge in testing demands is soon anticipated. Below, are two such regulatory changes that are poised to have an immediate impact:

- **Disclosing PFAS chemical releases from 2020**
  A new requirement under the US Toxic Release Inventory (TRI) Reporting Program will require companies to disclose the annual chemical releases of 172 PFAS to the EPA from 2020 onwards. The release data will subsequently be made available to the public.
  - What this means for PFAS testing labs: Now that companies need to report their PFAS chemical release data, along with the presence of PFAS in products, there will be an increased need for accurate PFAS testing. Those with global supply chains or working with collaborators that never had to evaluate PFAS levels in the past will soon be required to perform tests.

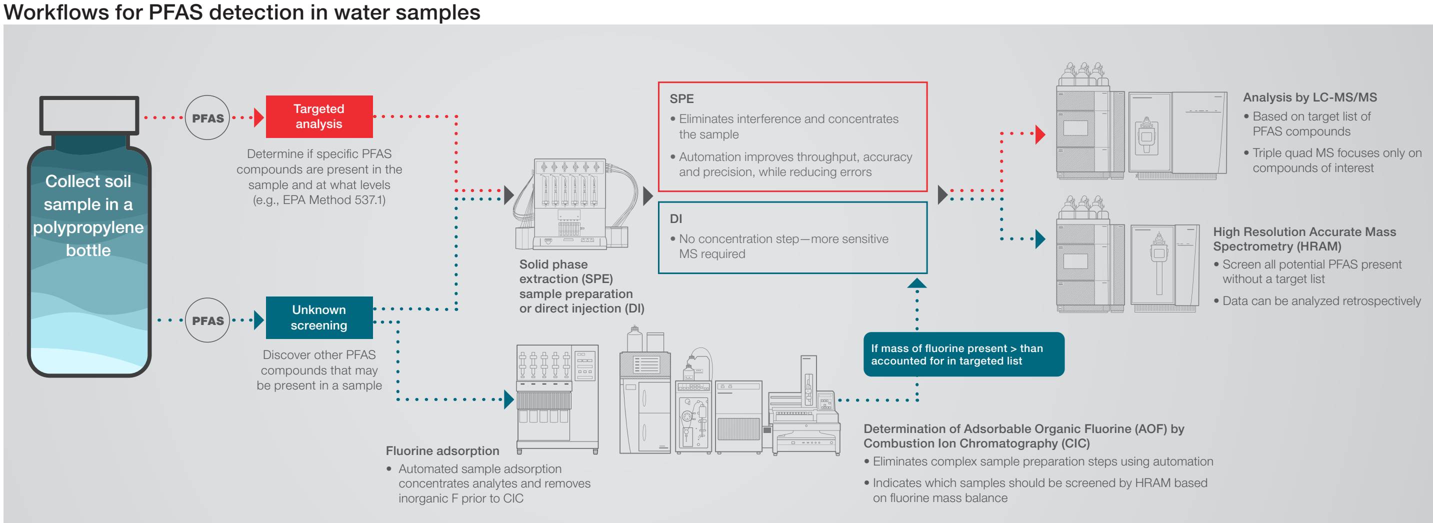
- **The fifth Unregulated Contaminant Monitoring Rule (UCMR 5) to be enforced in 2023**
  To monitor and determine the national occurrence of unregulated PFAS contaminants, the UCMR 5 requires public water systems to perform sample collection for 30 chemical contaminants between 2023 and 2025.
  - What this means for PFAS testing labs: As more public water systems will be required to collect samples, there will be an increased need for PFAS testing. Additionally, to cover the entire list of contaminants, testing laboratories are required to employ EPA-developed methods, pass proficiency tests, and get approved by the EPA for each method.

The growing interest in PFAS by the general public and government officials alike, along with improved scientific methods to identify new PFAS molecules, collectively influence regulatory decisions. Consequently, evolving regulatory requirements will continue to emerge in the short- to long-term future.

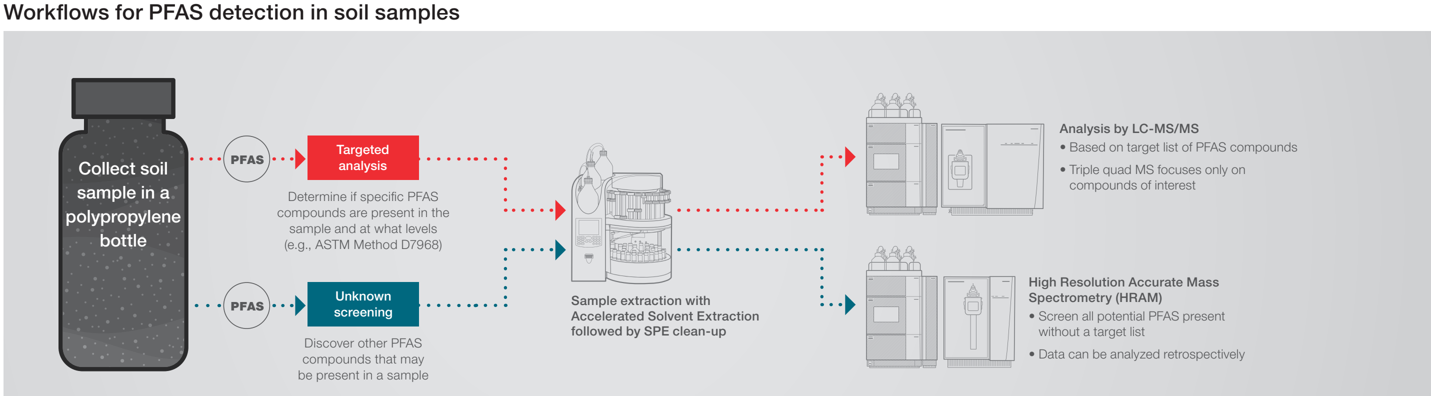
Workflow strategies for targeted and untargeted PFAS analysis

Based on the sample matrix being analyzed (for example, water or soil samples) and the goals of analysis, i.e., targeted analysis of known analytes or screening for unknowns, different PFAS workflows may be employed. Each workflow comprises fit-for-purpose technologies to extract, separate, detect and quantify analytes. The flowchart below provides a top-level summary of the different PFAS workflow possibilities for water and soil matrices, along with the recommended analytical method for each. Most PFAS workflows use LC for analyte separation while the mass spectrometer choice for detection and quantitation is driven by the PFAS analysis goal.

Workflows for PFAS detection in water samples



Workflows for PFAS detection in soil samples



There are core considerations for each stage of the process:

PFAS sampling: When collecting samples for PFAS analysis, care should be taken to not introduce any background PFAS into the sample, especially given that they can be found in laboratory consumables, gloves, waterproof materials, and blue ice packs used in shipping. To minimize contamination, the use of polypropylene sampling vessels to collect water and soil samples is recommended.

Sample preparation: Each matrix will require a different sample preparation technique. For water sample preparation, solid-phase extraction (SPE) is used for drinking water while dilution, filtration, and/or acidification steps are used for other non-potable water varieties. Soil samples, on the other hand, are prepared using accelerated solvent extraction (ASE) techniques.

To be able to handle an increasing number of samples, laboratories benefit from automating sample preparation steps to streamline workflows. One such platform is the Thermo Scientific™ Dionex™ AutoTrace™ instrument, which automates all four steps of solid-phase extraction – conditioning, loading, rinsing, and eluting – thereby boosting staff productivity and making protocols more reproducible. Additionally, this hands-free process also reduces the risk of background contamination as the flow paths are made of PFAS-free non-fluoropolymer materials.

Sample extraction for solid and semi-solid matrices, too, can be automated with accelerated solvent extractors that enable unattended extraction, filtration, and clean-up of multiple samples at a time. From a business perspective, automating sample preparation can be cost-effective in the longer term as it significantly reduces solvent consumption and cost per analysis by simultaneously processing multiple samples in only a few hours.

Automating sample preparation

	Solid-phase extraction	**Accelerated solvent extraction**
Water samples	Thermo Scientific™ Dionex™ AutoTrace™	
Soil samples		Thermo Scientific™ Accelerated Solvent Extractors

Targeted analysis of legacy and emerging PFAS

The most common PFAS tests involve quantifying known analytes outlined in the standardized EPA protocols. Customers requesting these routine tests on environmental samples are typically on time-sensitive schedules due to downstream manufacturing supply chains or, in many cases, have a sense of urgency due to potential public health repercussions. As such, laboratories conducing targeted PFAS analysis require validated methods and seamless workflows from sample preparation to data analysis. Introducing automation tools and specialized add-ons to these optimized workflows can reduce turnaround times and boost laboratory productivity.

A typical workflow for targeted PFAS analysis:

Drinking water/soil sample → Sample prep → LC separation and triple quadrupole MS/MS detection → Data processing and reporting

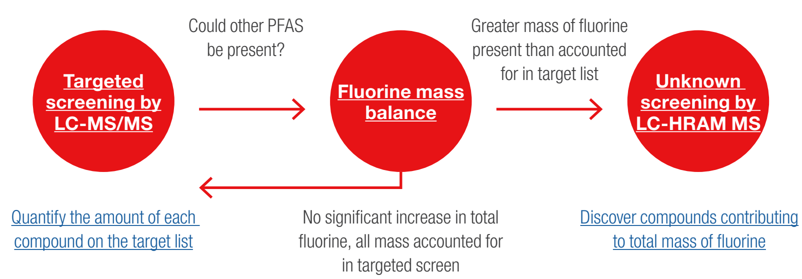
Instruments used in routine targeted PFAS analysis typically meet the following requirements:

- Robustness: Support high-throughput operations and require minimal downtime without compromising data quality
- Sensitivity: Meet regulatory detection limits or exceed these requirements
- Precision and accuracy: Provide reproducible, reliable data even with continuous use
- Scalable: Have the capacity to expand without requiring additional infrastructure

Non-targeted PFAS analysis: Screening or unknown profiling

Given the diversity of PFAS variants, it may be necessary to detect other PFAS analytes not on the target list to rule out public health concerns. Similarly, research teams performing environmental sampling studies may need to identify unregulated PFAS variants in the environment.

In these instances, the presence of possible unknown PFAS compounds is determined by measuring the overall fluorine mass in the sample, and then comparing that with the fluorine mass of known analytes. Fluorine mass is measured by detecting adsorbable organic fluorine (AOF) in the samples using combustion ion chromatography (CIC). If there is no significant increase in the fluorine mass, then there are no new PFAS compounds to profile (see workflow below). A difference in the fluorine mass balance, however, would mean the workflow can proceed to unknown screening using LC coupled with high-resolution accurate mass spectrometry (HRAM).



A typical workflow for untargeted PFAS screening and analysis: Drinking water/soil samples → Sample prep → [Targeted screening] LC separation and MS/MS analysis → Combustion ion chromatography → [Untargeted] HRAM → Data processing

For untargeted PFAS screening and profiling, instruments will need to meet the following requirements:

- High mass accuracy to generate reliable results
- Ultra high-resolution capability
- Retrospective analysis
- Minimal concern over false positives
- An accelerated path to confident results

Case study: Untargeted analysis to monitor PFAS pollutants in water sources

A research study at Duke University, USA, led by Dr. Lee Ferguson performed PFAS analysis on raw and drinking water samples collected from 405 sites across North Carolina to monitor known and unknown PFAS pollutants in the environment.

The results revealed that emerging unregulated PFAS compounds were present in environmental water sources at high concentrations, exceeding the Health Advisory levels. Currently not appearing on the EPA list of PFAS analytes, these findings bring attention to emerging PFAS variants and may even inform future regulations.

What's the best solution for PFAS analysis?

Choosing the most appropriate solutions largely depends on the type of PFAS screening or profiling analysis being performed – testing for known-knowns, known-unknowns or unknown-unknowns, or simply a mixture of it all. The overall business and research goals of the laboratory would also be a key determinant as some methods are more routine and repetitive, while others are investigative and open-ended.

Analysis type	MS Platform choice
• Routine targeted quantitation (known knowns)	TSQ Plus triple quadrupole mass spectrometers *Quantifies compounds that have been identified as important and provides sensitivity, selectivity, specificity, and robustness. Also enables low cost per sample or test.*
• Routine targeted quantitation (known knowns) • Untargeted screening (known unknowns)	Orbitrap Exploris HRAM mass spectrometers *Identifies and confirms if previously profiled analytes exist in samples. Also detects new compounds which may require identification and characterization.*
• Profiling (unknown unknowns)	Orbitrap Tribrid mass spectrometers *Harnesses the power of fine isotopic fidelity, performing deep MSn-level interrogation of each component by combining the best of quadrupole, Orbitrap, and linear ion trap mass analyzers. Ensures high levels of mass accuracy and sufficient sensitivity.*

	Combustion IC system	Triple quadrupole LC-MS	Orbitrap Exploris HRAM mass spectrometers	Orbitrap Tribrid mass spectrometers
Targeted quantitation		✓	✓	✓
Untargeted and targeted quantitation		✓	✓	✓
Targeted screening	Analysis of organofluorines	✓	✓	✓
Untargeted screening and identification			✓ (with Thermo Scientific™ AcquireX™ data acquisition workflow)	✓ (with AcquireX data acquisition workflow)
Retrospective analysis			✓	✓
Profiling of unknowns with simplicity				✓ (with AcquireX data acquisition workflow)

For routine targeted analyses of a predefined list of PFAS contaminants, triple quadrupole MS systems, such as the Thermo Scientific™ TSQ mass spectrometer series, would be most suitable. In these types of routine analyses, system robustness and higher throughput capabilities are key attributes to maintain optimal efficiency rates. Equally, high sensitivity to achieve the desired limits of detection as well as high specificity to confidently identify and quantify the target analytes are essential.

To enable comprehensive profiling of a vast range of PFAS analytes, especially when monitoring PFAS levels in the environment after a recent commercial activity or seasonal flooding, tandem MSn-level analyses may be required to ensure high standards of mass accuracy and sensitivity. The Thermo Scientific™ Orbitrap Tribrid™ mass spectrometers equipped with high mass range MSn support analyte discovery, profiling, and quantitation all on a single instrument.

When performing untargeted screening for previously profiled analytes or new PFAS variants (unknowns), MS platforms that enable identification and characterization need to be chosen. Quantitative HRAM instruments, such as the Thermo Scientific™ Orbitrap Exploris™ systems, are an ideal choice. The full-scan mode in HRAM systems also enables retrospective analyses, in case older sample records need to be re-analyzed through the lens of new regulatory changes.

As a general rule, to accommodate the increasing demands in PFAS testing and changing regulatory requirements, pick MS systems that serve the future needs and revenue goals of the laboratory rather than existing ones. Before choosing a system, consider factors such as instrument maintenance, training opportunities, technical support, software compatibility, ease of upgrading, and useful add-ons.

Streamlined data analysis and reporting

Having a suite of integrated data analysis software tools with automated processing and reporting capabilities improves efficiencies during routine sample analysis and boosts confidence in investigative “unknown” profiling. Typically purchased along with MS platforms, data analysis software systems come in application-specific packages to serve the unique requirements of a laboratory.

Implementing data automation into the overall workflow can set laboratories up for digital and remote operations. For instance, instruments can be remotely controlled through software settings, making it easier to manage everyday on-site activities and troubleshooting. Additionally, software tools can also improve data traceability as workflow details are meticulously recorded and stored in a centralized repository. When used along with spectral libraries for research projects, software systems can transform newly observed trends into actionable knowledge for the future.

Software tool	Application
Thermo Scientific™ TraceFinder™	• Simplifies high-throughput screening and quantitation • Increases laboratory output
Thermo Scientific™ mzCloud™ mass spectral library for PFAS identification	• To identify unknown compounds even when reference standards are unavailable • Improved confidence in unknown identification through comprehensive curation of all library content with extensive, fully annotated and searchable fragmentation spectra • Actionable results can be obtained even when there is no spectral library match • Searches can be performed for free both online and offline, or can be directly accessed through the Compound Discoverer software
Thermo Scientific™ Chromeleon™ chromatography data system software	• Compliance-ready control of instrumentation across multiple workflows • Customizable to meet user-specific requirements, regulatory guidelines, and reporting preferences
Thermo Scientific™ Compound Discoverer™ software	• To identify unknowns and examine important statistical differences between sample sets • Works with mzCloud library and other extensive online libraries

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Key considerations for PFAS accredited laboratories

Running fully validated PFAS protocols and obtaining reliable data are only the fundamental aspects of a high-performing analytical testing laboratory. To achieve accreditation for PFAS testing, and continue to hold that status, laboratories need to factor in numerous variables that stand the test of fast-paced operations, higher throughputs, and scaling up, all while upholding regulatory requirements.

The following checklist summarizes key considerations that can maximize performance and increase the revenue-generating potential of PFAS testing laboratories:

- ☐ Minimize cross-contamination by dedicating sample prep and clean-up space
- ☐ Meet or exceed current regulatory or advisory requirements
- ☐ Have a strategy for dealing with high concentration samples that fall outside the linear range
- ☐ Robust separation between linear and branched PFAS isomers
- ☐ Use standards from a second source or a different lot
- ☐ Deal with sample carryover effectively
- ☐ Ability to test a wide range of matrices
- ☐ Use gold-standard methods
- ☐ Have multiple accreditations across states or countries

While the above list isn't exhaustive, common themes that emerge for sustained success in PFAS testing are prioritizing data quality, maintaining compliance, and continually improving operational efficiencies.

Why PFAS testing laboratories need to future-proof themselves

There's more demand for PFAS testing today than ever before. With scientific understanding and global public awareness increasing, regulatory bodies are paying closer attention to the concerns surrounding these substances.

Simultaneously, analytical methods are becoming more advanced to support lower detection limits. Rather than being responsive to one-off incidents, research institutes are now being proactive, with dedicated teams continually sampling and monitoring PFAS variants in the environment, supported by government funding. It's safe to say that our knowledge of PFAS pollutants will only continue to expand, bringing changes to regulatory standards and introducing new variants to undertake.

With this much-needed collective focus on PFAS, there's also growing scrutiny on the testing methods being used and the quality of data subsequently being produced. Analytical laboratories need to prioritize both depth and breadth of capabilities to meet the lowest detection limits possible and reliably detect rare and unknown analytes. To future-proof themselves in the ever-evolving landscape of PFAS testing and stay ahead of competition, teams will need to invest in modern technologies and automation systems that can grow with their goals.

Thermo Fisher offers a suite of LC-MS instrumentation to support end-to-end PFAS analysis, along with automated sample preparation technologies and data analysis software. To request a demo, visit: Solutions for PFAS Testing | Thermo Fisher Scientific

Find out more at **thermofisher.com/pfas-testing**

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