



## TN-1362

# People and PFAS: Quantitation in Human Serum and Blood Using Volumetric Absorptive Microsampling (VAMS®)

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## Introduction

Bioaccumulation of per- and polyfluoroalkyl substances (PFAS) in the human body due to environmental exposure is a growing public health concern. PFAS are highly prevalent in the environment and in everyday consumer products, including our drinking water supply. Therefore, quantitative tools capable of accurately and precisely detecting low levels of PFAS in biological fluids are needed to understand the impact of PFAS on the human body.

Traditionally, human serum or plasma has been used to measure human exposure to PFAS. However, the process of collecting and analyzing these samples requires a phlebotomist to draw blood, a dedicated laboratory to separate serum or plasma from whole blood and the transportation of samples on dry ice with proper biohazard protocols. An alternative approach using dried blood spots (DBS) has been employed for several years to address these logistical challenges. However, there is a potential loss in accuracy associated with DBS due to the variability of blood volume on the filter paper and punch size.

An alternative approach to traditional blood sampling is the use of a Mitra® device powered by VAMS technology. These microsamples are collected with a hydrophilic polymer that provides a standardized 30 µL volume of whole blood for analysis. Due to the small volume collected by VAMS, analytical sensitivity is extremely important to quantify PFAS at the relevant levels for human health. The objective of this study was to compare the VAMS-derived PFAS values to traditional serum measurements using the SCIEX® 7500 system. The accuracy and robustness of each approach was assessed using both simulated and human-derived samples.

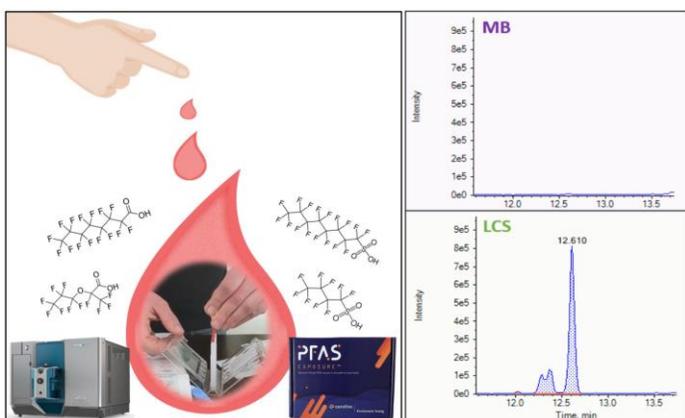
This technical note describes the trace level analysis of PFAS using only 60 µL of whole blood collected with VAMS devices (Figure 1). The sensitivity of the

SCIEX 7500 system was used to achieve serum detection limits ranging from 0.1 to 1.0 ng/mL, which are sufficient for PFAS biomonitoring in the general population. National Institute of Standards and Technology (NIST®) SRM 1957 samples that were collected using different sampling techniques were analyzed. These results demonstrated that samples collected with the VAMS method can be analyzed accurately and produce comparable results to samples collected by traditional PFAS serum sampling techniques. The use of VAMS for PFAS blood analysis overcomes many of the logistical obstacles common for traditional methods, such as the need for a trained phlebotomist for sample collection, sample storage and shipping costs.

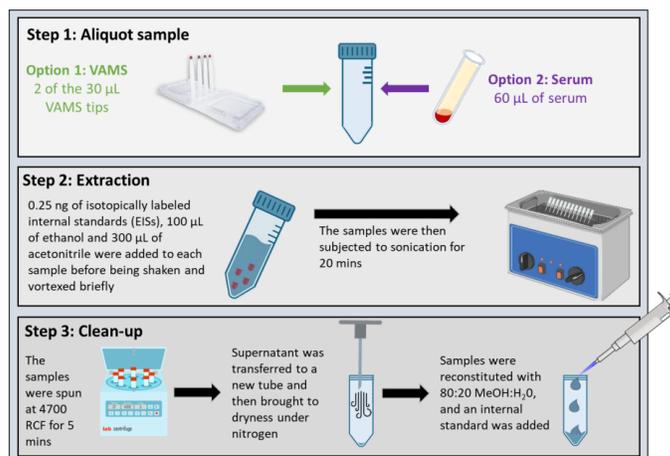
## Sample Preparation

The VAMS and serum samples were prepared for extraction separately, using a common method. The VAMS samples were measured by placing 2 of the VAMS tips containing 30 µL of sample in a polypropylene tube. In parallel, the serum samples were gently mixed and 60 µL was aliquoted into a separate polypropylene tube (Figure 2). From this point, the extractions followed identical procedures. Each sample was combined with 0.25 ng of isotopically labeled surrogates, 100 µL of Ethanol and 300 µL of Acetonitrile. The samples were then sonicated for 20 minutes and centrifuged for 5 minutes. The supernatant of each sample was separated and transferred to new polypropylene tubes. The remaining precipitates were combined with 300 µL of Methanol and shaken before undergoing centrifugation. The supernatants were separated and combined with the appropriate supernatant collected from the first extraction step. Finally, the samples were blown to dryness under Nitrogen and reconstituted using Methanol / Water (80:20, v/v) and an internal standard was added.

**Figure 1.** Graphical Abstract (Left) and Comparison of PFOS Content (Right) in the Method Blank Sample (Purple) and Laboratory Control Sample (Green).



**Figure 2.** Simplified Sample Extraction Procedure for Both VAMS and Serum Samples.



**LC Conditions**

**Column:** Gemini™ 3 µm C18  
**Dimensions:** 50 x 2.0 mm  
**Part No.:** [00B-4439-B0](#)  
**Trap Column:** Luna™ 3 µm NH<sub>2</sub>  
**Trap Column Dimensions:** 50 x 2.0 mm  
**Trap Column Part No.:** [00B-4377-B0](#)  
**Mobile Phase:** A: 20 mM Ammonium Acetate in Water  
 B: 10 mM Ammonium Acetate in Methanol  
**Gradient:**

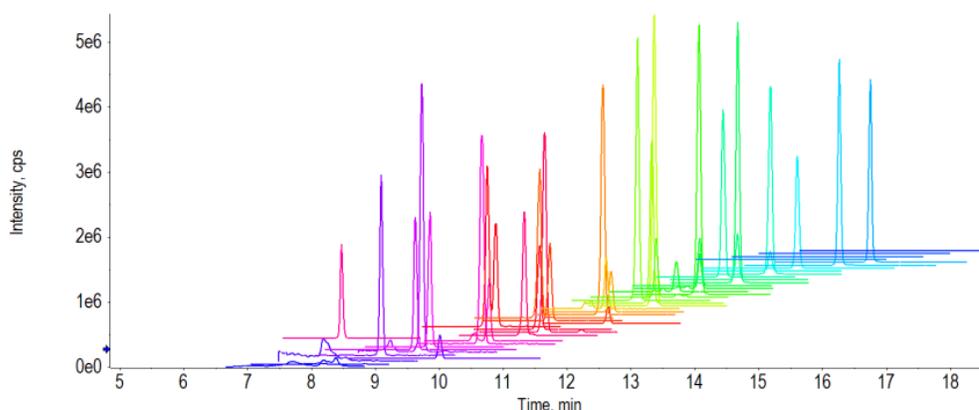
Time (min)	%B
0.00	10
5.00	10
5.10	45
12.0	80
12.1	99
14.0	99

**Flow Rate:** 0.5 mL/min  
**Injection Volume:** 10 µL  
**Temperature:** 25 °C  
**LC System:** SCIEX® ExionLC™  
**Detection:** MRM  
**Detector:** SCIEX 7500

**MRM Conditions**

**Ion Source:** ESI  
**Polarity:** Negative  
**Source Temperature:** 325 °C  
**GS1:** 35 psi  
**GS2:** 70 psi  
**CUR:** 40 psi  
**CAD:** 10 psi  
**IS:** -1500 V

**Figure 3.** Example Chromatogram Showing the PFAS Analyzed from a Laboratory Control Sample (LCS).

**Results and Discussion**

The method detection limits (MDLs) were consistent for both serum and VAMS® samples and were ≤0.5 ng/mL for each PFAS (**Table 1**). The MDLs were established following the guidelines set by the National Environmental Laboratory Accreditation Conference (NELAC) and 40 CFR Part 136.

The method described here involves processing a minimum of 7 spiked samples and 7 method blank samples. The samples designated for determining the MDL were prepared in 3 batches on separate calendar dates. These samples were then analyzed alongside 7 laboratory blanks. The MDLs for both serum and VAMS samples ranged from 0.05 to 0.16 ng/mL. The PFOA, PFOS, and PFHxS compounds had MDLs of 0.11 ng/mL, 0.081 ng/mL, and 0.05 ng/mL respectively.

Laboratory control samples (LCS) were aqueous solutions containing 4 % bovine serum albumin that were spiked with native and isotopically labeled PFAS standards. A LCS and a method blank (MB) sample were run every 20 samples. Recoveries ranged from 76 % to 112 % for the VAMS-collected samples, with an average recovery of 99% across all analytes (**Figure 4**). Recoveries for the serum samples, however, ranged from 69.7 % to

148.3 %, with an average recovery of 88.3 %. These results suggest that a VAMS-based approach might improve the efficiency of the extraction of PFAS compared to the serum-based approach. Further experiments would be needed to confirm this result.

An analysis of the NIST® SRM 1957 Organic Contaminants in Non-Fortified Human Serum was performed to compare the accuracy and precision attainable using different sample collection techniques. Aliquots of NIST SRM 1957 were collected for analysis using either VAMS devices or calibrated pipettes to simulate earlier described VAMS and serum samples, respectively (**Figure 5**). Each measurement was performed in triplicate. The average recovery of all PFAS in the simulated VAMS sample was 100.4 %, with a relative standard deviation (RSD) of 5.7 %. When the serum sample was simulated, 87.3 % of PFAS were recovered with an RSD of 2.6 %. It is important to note that 13C labeled internal standards were added prior to the extraction process and used for quantitation. However, the serum from NIST SRM 1957 was not added to the vacutainers prior to analysis.

To further assess analytical reproducibility, we calculated the coefficients of variation (CVs) for duplicate samples and determined the average for each sample type (**Figure 5**). Simulated serum samples (n = 10 PFAS) exhibited average CVs >20 % more frequently than simulated VAMS samples (n=3 PFAS). Notably, there were no discernible patterns indicating higher CVs for PFAS with higher or lower detection frequencies.

Have questions or want more details on implementing this method? We would love to help! Visit [www.phenomenex.com/Chat](http://www.phenomenex.com/Chat) to get in touch with one of our Technical Specialists

Table 1. Method Detection Limits (MDL) for PFAS in Serum and VAMS® Samples.

Compound Name	Acronym	MDL (ng/mL)	
		Serum	VAMS
<b>Perfluoroalkyl Carboxylic Acids (PFCAs)</b>			
Linear Perfluorooctanoic Acid	Linear PFOA	0.11	0.11
Branched Perfluorooctanoic Acid	Branched PFOA	0.11	0.11
Perfluorohexanoic Acid	PFHxA	0.27	0.27
Perfluoroheptanoic Acid	PFHpA	0.05	0.05
Perfluorononanoic Acid	PFNA	0.07	0.07
Perfluorodecanoic Acid	PFDA	0.07	0.07
Perfluoroundecanoic Acid	PFUnA	0.05	0.05
Perfluorododecanoic Acid	PFDoA	0.05	0.05
Perfluorotridecanoic Acid	PFTTrDA	0.05	0.05
Perfluorotetradecanoic Acid	PFTeA	0.05	0.05
Perfluorohexadecanoic Acid	PFHxDA	0.05	0.05
Perfluorooctadecanoic Acid	PFODA	0.061	0.061
<b>Perfluoroalkyl Sulfonic Acids (PFSA)</b>			
Perfluorobutanesulfonic Acid	PFBS	0.05	0.05
Perfluoropentanesulfonic Acid	PFPeS	0.05	0.05
Perfluorohexanesulfonic Acid	PFHxS	0.05	0.05
Perfluoroheptanesulfonic Acid	PFHpS	0.05	0.05
Linear Perfluorooctanesulfonic Acid	Linear PFOS	0.081	0.081
Branched Perfluorooctanesulfonic Acid	Branched PFOS	0.081	0.081
Perfluorononanesulfonic Acid	PFNS	0.08	0.08
Perfluorodecanesulfonic Acid	PFDS	0.05	0.05
Perfluorododecanesulfonic Acid	PFDoS	0.05	0.05
Perfluoroethylcyclohexane Sulfonic Acid	PFECHS	0.05	0.05
<b>Fluorotelomer Sulfonic Acids</b>			
4:2 Fluorotelomer Sulfonic Acid	4:2 FTS	0.05	0.05
6:2 Fluorotelomer Sulfonic Acid	6:2 FTS	0.17	0.17
8:2 Fluorotelomer Sulfonic Acid	8:2 FTS	0.05	0.05
10:2 Fluorotelomer Sulfonic Acid	10:2 FTS	0.05	0.05
<b>Fluorotelomer Phosphate Diesters</b>			
Bis(1H, 1H,2H,2H-Perfluorooctyl) Phosphate	6:2 diPAP	0.16	0.16
Bis(1H, 1H,2H,2H-Perfluorodecyl) Phosphate	8:2 diPAP	0.06	0.06
Bis(1H, 1H,2H,2H-Perfluoroundecyl) Phosphate	10:2 diPAP	0.05	0.05
(1H, 1H,2H,2H-Perfluorooctyl)-1H,1H,2H,2H-Perfluorodecyl) Phosphate	6:2/8:2 diPAP	0.07	0.07
<b>Perfluoroalkyl Sulfonamides</b>			
Perfluorooctanesulfonamidoacetic Acid	FOSA	0.05	0.05
N-Methylperfluoro-1-Octanesulfonamide	NMeFOSAA	0.05	0.05
N-Ethyl Perfluorooctanesulfonamidoacetic Acid	NEFOSAA	0.06	0.06
<b>Fluorotelomer Carboxylic Acid</b>			
7:3 Fluorotelomer Carboxylic Acid	7:3 FTCA	0.06	0.06
<b>Chlorinated Perfluoroether Sulfonic Acid</b>			
11-Chloroeicosafuoro-3-Oxaundecane-1-Sulfonic Acid	11Cl PF3OUdS	0.05	0.05
9-Chlorohexadecafluoro-3-Oxanonane-1-Sulfonic Acid	9Cl PF3ONS	0.05	0.05
<b>Fluoroether Carboxylic and Sulfonic Acids</b>			
4,8-Dioxa-3H-Perfluorononanoate	ADONA	0.05	0.05
Perfluoro-2-Propoxypropanoic Acid	GenX	0.05	0.05
2,2,3,3-Tetrafluoro-3-((1,1,1,2,3,3-Hexafluoro-3-(1,2,2,2-Tetrafluoroethoxy)Propan-2-yl)Oxy)Propanoic Acid	Hydro EVE Acid	0.05	0.05
Perfluoro-2-[[Perfluoro-3-(Perfluoroethoxy)-2-Propanyl]Oxy]Ethanesulfonic Acid	Hydro PS Acid	0.05	0.05
Nonafluoro-3,6-Dioxaheptanoic Acid	NFDHA	0.056	0.056
Perfluoro(2-Ethoxyethane)Sulphonic Acid	PFEESA	0.05	0.05
Perfluoropolyether	PFPE 1	0.05	0.05
Perfluoro-4-Methoxybutanic Acid	PFMBA	0.05	0.05
1,1,2,2-Tetrafluoro-2-[1,2,2,3,3-Pentafluoro-1-(Trifluoromethyl)Propoxy] Ethanesulfonic Acid	R PSDCA	0.05	0.05



Figure 4. Compiled Laboratory Control Samples (LCS) Data for VAMS® Analytical Batches.

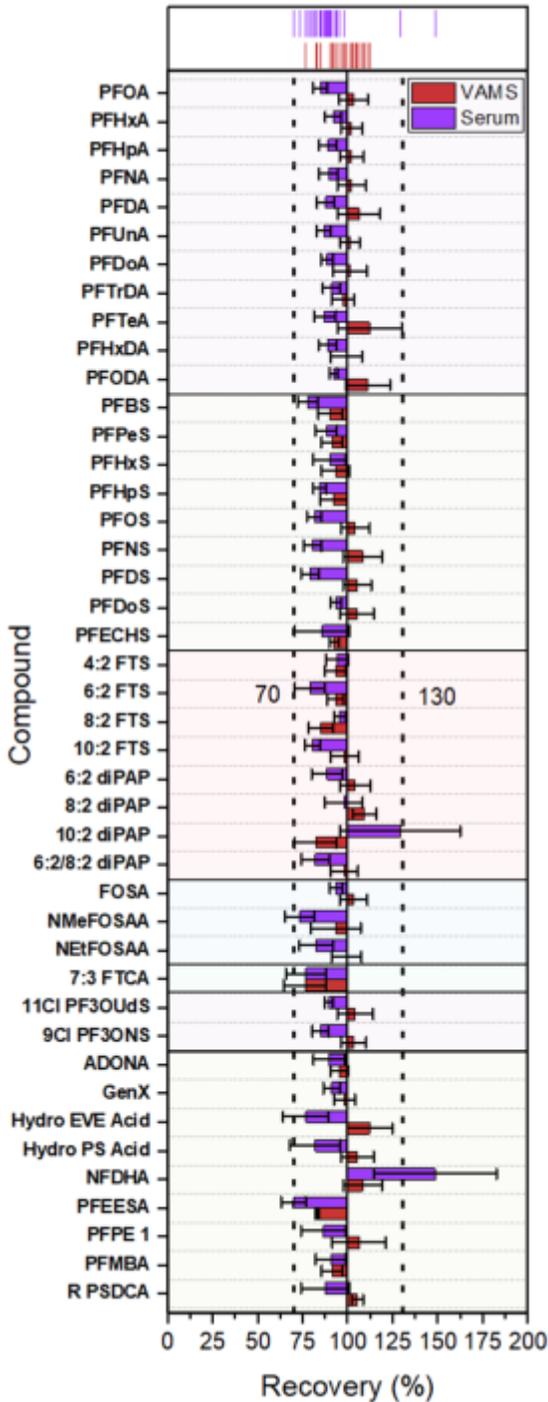
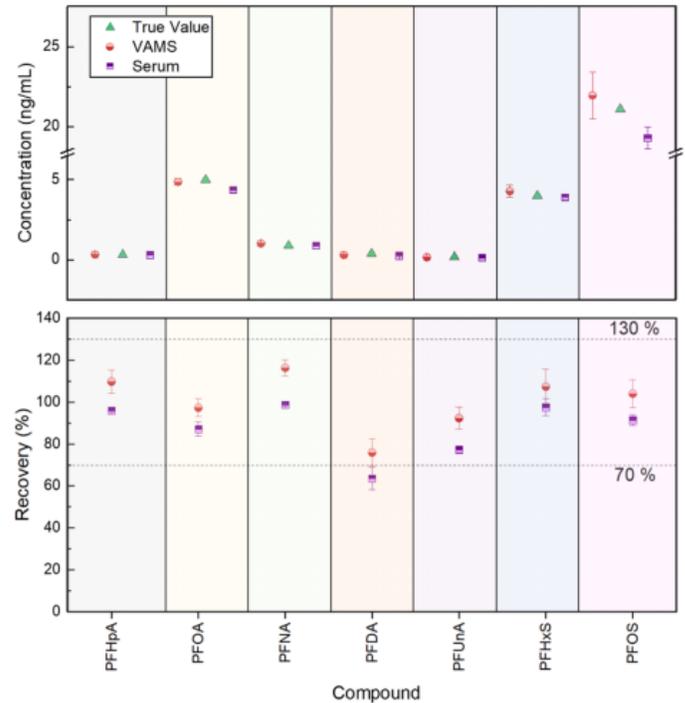


Figure 5. Evaluation of NIST® SRM 1957.



**Conclusion**

The evaluation of the NIST SRM 1957 was conducted in triplicate using both the serum and VAMS collection methods. This experiment demonstrated consistent outcomes between the 2 approaches. The VAMS collection method exhibited greater accuracy, as evidenced by an average recovery of approximately 100 % for the reported compounds. In contrast, the serum collection method displayed greater precision (RSD <3 %) compared to the VAMS method (RSD <6 %).

The analytical method proved to be highly sensitive even with a small blood volume. The detection limits achieved through serum conversion, which ranged from 0.1 to 1.0 ng/mL, are adequately sensitive for biomonitoring purposes in the general population. This is particularly evident as median serum concentrations >1.0 ng/mL were observed for only 6 of the 15 investigated PFAS. Regardless, generating additional data using this method in conjunction with traditional serum measurements will help to understand the potential differential partitioning of emerging PFAS in serum, plasma and whole blood samples.



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3 μm Microbore and Minibore Columns (mm)								SecurityGuard™ Cartridges (mm)			
Phases	50 x 1.0	150 x 1.0	30 x 2.0	30 x 2.1	50 x 2.0	50 x 2.1	100 x 2.0	100 x 2.1	150 x 2.0	150 x 2.1	4 x 2.0* /10 pk
Silica(2)	—	<a href="#">00F-4162-AO</a>	—	—	<a href="#">00B-4162-BO</a>	—	<a href="#">00D-4162-BO</a>	—	<a href="#">00F-4162-BO</a>	—	<a href="#">AJ0-4347</a>
C8(2)	—	—	<a href="#">00A-4248-BO</a>	—	<a href="#">00B-4248-BO</a>	—	<a href="#">00D-4248-BO</a>	—	<a href="#">00F-4248-BO</a>	—	<a href="#">AJ0-4289</a>
C18(2)	<a href="#">00B-4251-AO</a>	<a href="#">00F-4251-AO</a>	<a href="#">00A-4251-BO</a>	—	<a href="#">00B-4251-BO</a>	—	<a href="#">00D-4251-BO</a>	—	<a href="#">00F-4251-BO</a>	—	<a href="#">AJ0-4286</a>
CN	—	—	—	—	<a href="#">00B-4254-BO</a>	—	<a href="#">00D-4254-BO</a>	—	<a href="#">00F-4254-BO</a>	—	<a href="#">AJ0-4304</a>
Phenyl-Hexyl	—	—	—	—	<a href="#">00B-4256-BO</a>	—	<a href="#">00D-4256-BO</a>	—	<a href="#">00F-4256-BO</a>	—	<a href="#">AJ0-4350</a>
NH <sub>2</sub>	—	<a href="#">00F-4377-AO</a>	<a href="#">00A-4377-BO</a>	—	<a href="#">00B-4377-BO</a>	—	<a href="#">00D-4377-BO</a>	—	<a href="#">00F-4377-BO</a>	—	<a href="#">AJ0-4301</a>
HILIC	—	—	—	—	<a href="#">00B-4449-BO</a>	—	<a href="#">00D-4449-BO</a>	—	<a href="#">00F-4449-BO</a>	—	<a href="#">AJ0-8328</a>
PFP(2)	—	<a href="#">00F-4447-AO</a>	<a href="#">00A-4447-BO</a>	—	<a href="#">00B-4447-BO</a>	—	<a href="#">00D-4447-BO</a>	—	<a href="#">00F-4447-BO</a>	—	<a href="#">AJ0-8326</a>
Polar Pesticides	—	—	—	<a href="#">00A-4798-AN</a>	—	<a href="#">00B-4798-AN</a>	—	<a href="#">00D-4798-AN</a>	—	<a href="#">00F-4798-AN</a>	<a href="#">AJ0-8789</a>

For ID: 2.0-3.0 mm

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Phases	50 x 1.0	20 x 2.0	30 x 2.0	50 x 2.0	100 x 2.0	150 x 2.0	50 x 3.0	100 x 3.0	150 x 3.0	4 x 2.0* /10 pk
C18	<a href="#">00B-4439-AO</a>	<a href="#">00M-4439-BO</a>	<a href="#">00A-4439-BO</a>	<a href="#">00B-4439-BO</a>	<a href="#">00D-4439-BO</a>	<a href="#">00F-4439-BO</a>	<a href="#">00B-4439-YO</a>	<a href="#">00D-4439-YO</a>	<a href="#">00F-4439-YO</a>	<a href="#">AJ0-7596</a>
C6-Phenyl	—	—	—	<a href="#">00B-4443-BO</a>	<a href="#">00D-4443-BO</a>	<a href="#">00F-4443-BO</a>	<a href="#">00B-4443-YO</a>	<a href="#">00D-4443-YO</a>	<a href="#">00F-4443-YO</a>	<a href="#">AJ0-7914</a>
NX-C18	<a href="#">00B-4453-AO</a>	<a href="#">00M-4453-BO</a>	<a href="#">00A-4453-BO</a>	<a href="#">00B-4453-BO</a>	<a href="#">00D-4453-BO</a>	<a href="#">00F-4453-BO</a>	<a href="#">00B-4453-YO</a>	<a href="#">00D-4453-YO</a>	<a href="#">00F-4453-YO</a>	<a href="#">AJ0-8367</a>

For ID: 2.0-3.0 mm

\*SecurityGuard Analytical Cartridges require holder, Part No.: [KJ0-4282](#)

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Description	Part No.	Unit
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Strata™-X-AW 33 μm, Polymeric Weak Anion-PFAS, 200 mg / 6 mL	<a href="#">8B-S541-FCH</a>	30/pk
Strata-X-AW 33 μm, Polymeric Weak Anion-PFAS, 150 mg / 6 mL	<a href="#">8B-S541-SCH</a>	30/pk
Strata-X-AW 33 μm, Polymeric Weak Anion-PFAS, 500 mg / 6 mL	<a href="#">8B-S541-HCH</a>	30/pk
Strata PFAS (WAX/GCB), 200 mg / 50 mg / 6 mL	<a href="#">CS0-9207</a>	30/pk
Strata PFAS (WAX/GCB), 500 mg / 50 mg / 6 mL	<a href="#">CS0-9208</a>	200/pk
Strata PFAS (WAX/GCB), 250 mg / 50 mg / 6 mL	<a href="#">CS0-9215</a>	200/pk
Strata PFAS (GCB/WAX), 50 mg / 200 mg / 6 mL	<a href="#">CS0-9214</a>	30/pk
Strata PFAS (GCB/WAX), 250 mg / 100 mg / 6 mL	<a href="#">CS0-9217</a>	30/pk
<b>SecurityCAP™ Safety Filters</b>		
SecurityCAP mobile phase safety filter for PFAS testing, 6-month capacity, 1/4 in-28 thread	<a href="#">AC2-0961-P</a>	10/pk
SecurityCAP mobile phase starter kit for PFAS testing, 3-port GL45 caps and 6-month safety filter	<a href="#">AC2-4345-P</a>	Ea
SecurityCAP mobile phase starter kit for PFAS testing, 2-port GL45 caps and 6-month safety filter	<a href="#">AC2-4245-P</a>	Ea
<b>Vials</b>		
Verex™ 9 mm Black PP cap, preslit w/ blue silicone/clear PP liner	<a href="#">ARO-89P7-12</a> <a href="#">ARO-89P7-13</a>	100/pk 1000/pk
Verex 9 mm Black PP cap, nonslit w/ blue silicone/clear PP liner	<a href="#">ARO-89N7-12</a> <a href="#">ARO-89N7-13</a>	100/pk 1000/pk
Verex 9 mm PP vial, clear, 300 μL	<a href="#">ARO-39P2-13</a>	1000/pk
Verex 9mm PP vial, clear, 700 μL	<a href="#">ARO-39P1-13</a>	1000/pk
Verex 9mm PP vial, clear, 1.7 mL	<a href="#">ARO-39P0-13</a>	1000/pk

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