

Supplementary Table 1: Calibration levels and Spiking Concentrations

Concentration in 50:50 Acetonitrile:MilliQ™ Water with phosphoric acid (nominal ng mL ⁻¹)													
Standard	1	2	3	4	5	6	7	8	9	10	11	12	13
All PFCAs, PFSA's (except PFOS), EtFOSAA, MeFOSAA, FOSA, FBSA and SRSs	0.00250	0.00500	0.0100	0.0250	0.0500	0.100	0.250	0.500	1.00	2.50	5.00	10.0	25.0
PFOS	0.0100	0.0200	0.0400	0.100	0.200	0.400	1.00	2.00	4.00	10.0	20.0	40.0	100

a) IS concentration in all standards was 9.25 ng/mL⁻¹ for the method validation and 1.00 ng/mL⁻¹ for the ARC study

Concentration in human plasma; 9.63 dilution factor applied (nominal ng mL ⁻¹)													
All PFCAs, PFSA's (except PFOS), EtFOSAA, MeFOSAA, FOSA, FBSA and SRSs	0.0241	0.0482	0.0963	0.241	0.482	0.963	2.41	4.82	9.63	24.1	48.2	96.3	241
PFOS	0.0963	0.193	0.385	0.963	1.93	3.85	9.63	19.3	38.5	96.3	193	385	963

Method Validation LCS Spiking Levels

Bovine LCS (prepared in triplicate)	Concentration in Serum (ng mL ⁻¹)			
	Level 1	Level 2	Level 3	Level 4
All PFCAs, PFSA's (except PFOS), EtFOSAA, MeFOSAA, FOSA, FBSA and SRSs	0.500	5.00	25.0	35.0
PFOS	2.00	20.0	100	140

Human LCS (prepared in triplicate)	Concentration in Plasma (ng mL ⁻¹)		
	Level 1	Level 2	Level 3
All PFCAs, PFSA's (except PFOS), EtFOSAA, MeFOSAA, FOSA, FBSA and SRSs	5.00	25.0	35.0
PFOS	20.0	100	140

Human B+L LCSs	Concentration in Plasma (ng mL ⁻¹)	
	Level 1	Level 2
PFOA, PFHS and SRSs	5.00	25.0
PFOS	20.0	100

Supplementary Table 1 (continued)**American Red Cross LCS Spiking Levels**

Bovine LCS (prepared in triplicate)	Concentration in Serum (ng mL ⁻¹)		
	Level 1	Level 2	Level 3
All PFCAs, PFSA's (except PFOS), EtFOSAA, MeFOSAA, FOSA, FBSA and SRSs	0.250	2.50	17.5
PFOS	1.00	10.0	70.0

Human LCS (prepared in triplicate)	Concentration in Plasma (ng mL ⁻¹)		
	Level 1	Level 2	Level 3
All PFCAs, PFSA's (except PFOS), EtFOSAA, MeFOSAA, FOSA, FBSA and SRSs	2.50	17.5	175
PFOS	10.0	70.0	700

Human B+L LCSs (prepared in triplicate)	Concentration in Plasma (ng mL ⁻¹)	
	Level 1	Level 2
PFOA, PFHS and SRSs	2.50	10.0
PFOS	10.0	40.0

Method Blank and Sample SRS and IS Spike Levels

	Method Validation (ng/mL ⁻¹ in serum)	American Red Cross (ng/mL ⁻¹ in Serum)
IS	17.5	1.00
SRS	1.00	1.00

Supplementary Table 2 Method analytes, internal standards, and surrogate recovery standards

Compound	Description	MRM mass transition (s)^a
PFBA (C4 Acid)	Method analyte	213 > 169
PFPeA (C5 Acid)	Method analyte	263 > 219
PFHxA (C6 Acid)	Method analyte	313 > 269, 313 > 119
PFHpA (C7 Acid)	Method analyte	363 > 319, 363 > 169
PFOA (C8 Acid) (Branched and Linear)	Method analyte	413 > 369, 413 > 219, 413 > 169
PFNA (C9 Acid)	Method analyte	463 > 419, 463 > 219, 463 > 169
PFDA (C10 Acid)	Method analyte	513 > 469, 513 > 219
PFUnA (C11 Acid)	Method analyte	563 > 519, 563 > 269
PFDaA (C12 Acid)	Method analyte	613 > 569, 613 > 219, 613 > 169
PFBS (C4 Sulfonate)	Method analyte	299 > 99, 299 > 80
PFHS (C6 Sulfonate) (Branched and Linear)	Method analyte	399 > 99, 399 > 80
PFOS (C8 Sulfonate) (Branched and Linear)	Method analyte	499 > 130, 499 > 99, 499 > 80
FOSA (C8 Sulfonamide)	Method analyte	498 > 78
MeFOSAA	Method analyte	570 > 169, 570 > 219, 570 > 83
EtFOSAA	Method analyte	584 > 169, 584 > 219, 584 > 83
FBSA (C4 Sulfonamide)	Method analyte	298 > 78
Internal standards and surrogate recovery standards		
[1,2,3- ¹³ C ₃]PFBA	SRS representing C4-C5 PFCAs	216 > 172
[1,2,3,4- ¹³ C ₄]PFOA	SRS representing C6-C8 PFCAs	417 > 372
[1,2- ¹³ C ₂]PFUnA	SRS representing C9-C12 PFCAs	565 > 520
[1,2,3,4- ¹³ C ₄]PFOS	SRS representing all PFSAs, FBSA, FOSA, EtFOSAA, MeFOSAA	503 > 80
[1,2,3,4- ¹³ C ₄]PFBA	IS PFBA, (1,2,3- ¹³ C ₃)PFBA SRS	217 > 172
[1,2,3,4,5- ¹³ C ₅]PFPeA	IS PFPeA	268 > 223
[1,2- ¹³ C ₂]PFHxA	IS PFHxA	315 > 270
[1,2,3,4]- ¹³ C ₂]PFHpA	IS PFHpA	367 > 322
[¹³ C ₈]PFOA	IS PFOA, [1,2,3,4- ¹³ C ₄]PFOA SRS	421 > 376
[1,2,3,4,5- ¹³ C ₅]PFNA	IS PFNA	472 > 427
[1,2- ¹³ C ₂]PFDA	IS PFDA	519 > 474
[1,2,3,4,5,6,7- ¹³ C ₇]PFUnA	IS PFUnA, [1,2- ¹³ C ₂]PFUnA SRS	570 > 525
[1,2- ¹³ C ₂]PFDaA	IS PFDaA	615 > 570
[¹⁸ O ₂]PFBS	IS PFBS, FBSA	303 > 84

Supplementary Table 2 Method analytes, internal standards, and surrogate recovery standards

Compound	Description	MRM mass transition (s)^a
[1,2,3- ¹³ C ₃]PFHS	IS PFHS	402 > 80
[¹³ C ₈]PFOS	IS PFOS, [1,2,3,4- ¹³ C ₄]PFOS SRS	507 > 80
[[1,2,3,4,5,6,7,8- ¹³ C ₈]FOSA	IS FOSA	506 > 78
<i>d</i> 3-MeFOSAA	IS MeFOSAA	573 > 169, 573 > 219, 573 > 83
<i>d</i> 5-EtFOSAA	IS EtFOSAA	589 > 169, 589 > 219, 589 > 83

^a The individual transitions were summed to produce a SRM plot, which was used for quantitation.

Supplementary Figure 1: Dilution Factor Calculation

$$9.63 = \frac{\text{total extract (transferred extract + 5\% H}_3\text{PO}_4), \text{ mL}}{\text{transferred volume of extract, mL}} \times \frac{\text{total extract volume from PPT step, mL}}{\text{volume of serum used, mL}}$$

Supplementary Table 3. QC analyte and SRS Recovery Results from American Red Cross Study

Target Analyte	Mean Recover y	% RSD (n)	Method Uncertainty
Bovine Serum QCs (Fortified with Linear Reference Substances and SRSs)			
PFHxA (C ₆ PFCA)	95.4%	5.1 (171)	<u>9.6%</u>
PFHpA (C ₇ PFCA)	94.9%	3.9 (171)	<u>7.4%</u>
PFOA (C ₈ PFCA)	88.3%	9.0 (171)	<u>16%</u>
PFNA (C ₉ PFCA)	96.3%	5.3 (171)	<u>10%</u>
PFDA (C ₁₀ PFCA)	94.6%	10 (171)	<u>19%</u>
PFUnA (C ₁₁ PFCA)	93.5%	6.8 (168)	<u>13%</u>
PFDoA (C ₁₂ PFCA)	92.9%	14 (171)	<u>26%</u>
PFBS (C ₄ PFSA)	93.9%	3.4 (171)	<u>6.3%</u>
PFHS (C ₆ PFSA)	95.5%	4.0 (171)	<u>7.6%</u>
PFOS (C ₈ PFSA)	99.0%	8.0 (171)	<u>16%</u>
FOSA	95.2%	4.1 (171)	<u>7.8%</u>
MeFOSAA	87.4%	16 (171)	<u>28%</u>
EtFOSAA	85.7%	17 (171)	<u>28%</u>
[1,2,3,4- ¹³ C ₄]PFOA	96.3%	8.8 (189)	<u>17%</u>
[1,2- ¹³ C ₂]PFUnA	97.3%	9.7 (198)	<u>19%</u>
[1,2,3,4- ¹³ C ₄]PFOS	96.1%	9.0 (180)	<u>17%</u>
Human Plasma QCs (Fortified with Linear Reference Substances and SRSs)			
PFHxA (C ₆ PFCA)	93.9%	3.1 (171)	<u>5.8%</u>
PFHpA (C ₇ PFCA)	94.1%	3.2 (174)	<u>6.0%</u>
PFOA (C ₈ PFCA)	92.5%	8.1 (126)	<u>15%</u>
PFNA (C ₉ PFCA)	94.7%	5.9 (168)	<u>11%</u>
PFDA (C ₁₀ PFCA)	93.1%	4.8 (168)	<u>8.9%</u>
PFUnA (C ₁₁ PFCA)	93.8%	4.9 (177)	<u>9.0%</u>
PFDoA (C ₁₂ PFCA)	93.4%	3.6 (153)	<u>6.6%</u>
PFBS (C ₄ PFSA)	93.5%	3.4 (174)	<u>6.3%</u>
PFHS (C ₆ PFSA)	95.2%	5.8 (165)	<u>11%</u>
PFOS (C ₈ PFSA)	95.6%	9.2 (174)	<u>17%</u>
FOSA	94.7%	4.1 (175)	<u>7.7%</u>
MeFOSAA	83.8%	12 (153)	<u>21%</u>
EtFOSAA	83.9%	14 (153)	<u>23%</u>
Human Plasma QCs (Fortified with Technical Reference Substances)			
PFOA (Technical)	83.7%	7.2 (114)	<u>12%</u>
PFHS (Technical)	101%	5.0 (114)	<u>10%</u>
PFOS (Technical)	102%	5.8 (108)	<u>12%</u>

Human Plasma QCs (SRSs) [d]

[1,2,3,4- ¹³ C ₄]PFOA	87.8%	7.5 (324)	<u>13%</u>
[1,2- ¹³ C ₂]PFUnA	89.2%	8.2 (339)	<u>14%</u>
[1,2,3,4- ¹³ C ₄]PFOS	87.5%	7.7 (309)	<u>13%</u>

American Red Cross Adult Blood Donor Samples (SRS Recoveries)

[1,2,3,4- ¹³ C ₄]PFOA	89.0%	7.3 (736)	± 13% [a]
[1,2- ¹³ C ₂]PFUnA	91.8%	10 (736)	± 19% [b]
[1,2,3,4- ¹³ C ₄]PFOS	89.4%	7.9 (736)	± 14% [c]

[a] Value is estimated data uncertainty for shorter chain length PFCAs: PFHxA, PFHpA, PFOA and PFNA in samples

[b] Value is estimated data uncertainty for long chain PFCAs: PFDA, PFUnA and PFDoA in samples

[c] Value is estimated data uncertainty for sulfonates/sulfonamides: PFBS, PFHS, PFOS, FOSA, MeFOSAA and EtFOSAA

[d] Values derived from both human plasma QC fortified with all analytes and human plasma QC fortified with only Technical Reference Substances.

Uncertainty values calculated according to A2LA guidance document "G104-Guide for Estimation of Measurement Uncertainty in Testing-December 4, 2014". Uncertainty is expressed as X (1±kσ); where X is the measured result k is the k-factor at the 95% confidence level and σ is the standard deviation.

Supplementary Table 4: NIST SRM 1957 Results

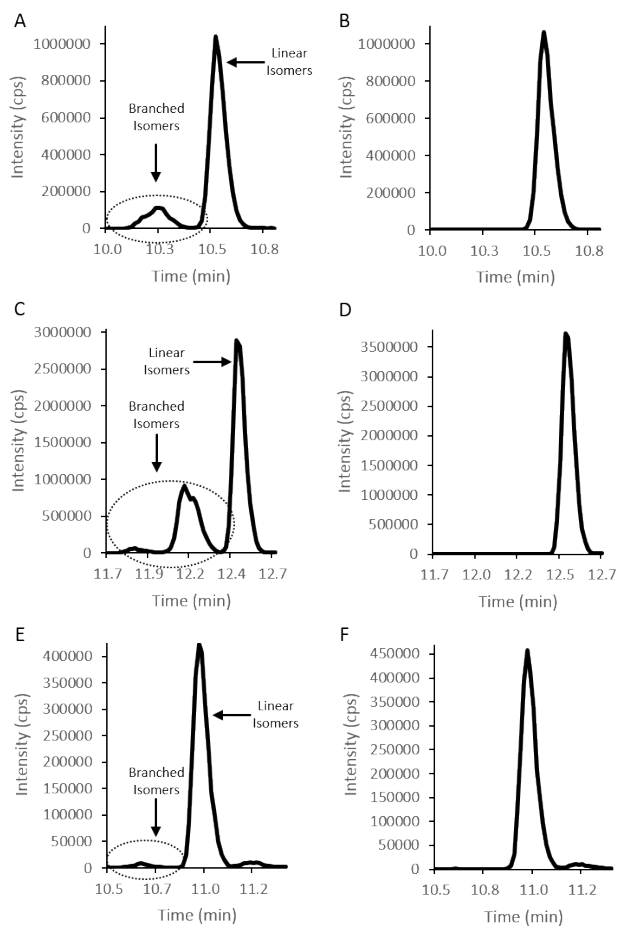
Analyte	Average Concentration (ng mL ⁻¹)	% RSD	n ^[a]	NIST SRM 1957 Ref. Value(ng mL ⁻¹)	Average Accuracy (%)
PFOA	4.16	13%	18	5.00	83.2%
PFOS	21.5	8.7%	18	21.1	102%
PFHS	3.58	12%	18	4.00	89.5%

[a] Values are from 6 separate extractions, each time prepared in triplicate.

Supplementary Table 5. LLOQs

Analyte	Method Validation LLOQ Achieved (ng mL ⁻¹)	LLOQs Achieved for American Red Cross for 21 batches (ng mL ⁻¹ in Serum/Plasma)	Number of American Red Cross Samples that were below the LLOQ (out of 736)
PFBA	4.82	ND	ND
PFPeA	0.0963	ND	ND
PFBS	0.0482	0.0232, 0.0465	679
PFHxA	0.0481	0.0232, 0.0932	681
PFHpA	0.0482	0.0466, 0.0932	643
PFHS	0.0482	0.0233, 0.0466	2
PFOA	0.0963	0.0232, 0.0465, 0.0932	2
PFNA	0.0482	0.0233, 0.0466, 0.0932	2
PFOS	0.193	0.0932, 0.186, 0.372	0
PFDA	0.0482	0.0233, 0.0466, 0.0932	21
FBSA	0.0481	ND	ND
PFUnA	0.0963	0.0466, 0.0932, 0.233	381
PFDoA	0.240	0.0466, 0.0932, 0.233	732
MeFOSAA	0.240	0.0232, 0.0465, 0.232	190
EtFOSAA	0.0481	0.0232, 0.0465, 0.0932, 0.232	656
FOSA	0.0240	0.0232, 0.0932	731
[1,2,3- ¹³ C ₃]PFBA	0.0959	ND	ND
[1,2,3,4- ¹³ C ₄]PFOA	0.0963	0.0232, 0.0465, 0.0932	NA
[1,2- ¹³ C ₂]PFUnA	0.0963	0.0232, 0.0465, 0.0932	NA
[1,2,3,4- ¹³ C ₄]PFOS	0.0920	0.0222, 0.0446, 0.0890	NA

ND; Analytes were not analyzed in Study



Supplementary Figure 2: Branched vs. Linear Isomers. A. TECHNICAL-PFHS (18.9% Branched) B. L-PFHS C. TECHNICAL-PFOS (30.1% Branched) D. L-PFOS E. TECHNICAL PFOA (4.7% Branched) F. L-PFOA.