Additional Information			
	Part 1	Part 2	Part 3
Sample pretreatment		Acid treatment, sonication	Precipitation of proteins with methanol
Extraction technique:	Ion Pair	SPE	on-line SPE using Betasil C8 (10mmx4mmx5µm)
Extraction solvents:	0.5 M tetrabutylammonium hydrogen sulfate (TBA) 0.25 M natrium carbonat/natrium bicarbonat buffer MTBE	MeOH	methanol
Clean Up:		SPE	on-line SPE using Betasil C8
LC column:	Betasil C18 2.1 x 50 mm, 5 µm	C18 7.5cm, 2.1mm i.d. Up to 90% ACN and 12.1mM NH4OAc in 0.1% AcOH	Betasil C8 (50mmx2.1mmx3µm) 15mM ammonium acetate in water - methanol (starting conditions 90:10 water:methanol final conditions 10:90 water:methanol)
LC/MS(MS):	API 2000 (Sciex)	Waters, MS/MS ESI-	Thermo Finnigan, TSQ quantum, triple quadrupole, ESI
(Labelled) Internal Standards: (Labelled) Recovery Standards: Results Corrected for Recovery: Results Corrected for Blanks:	3	3 2 Yes No	8 (MPFBA, MPFHxA, MPFOA, MPFNA, MPFDA, MPFDoDA, MPFHxS, MPFOS) not applicable yes, corrected by addition of internal standards no
Standard Method:			Method published: Haug et al. Journal of Chromatography A, 2009, 1216, p 385
Comments:			Concentration of PFCs in the standard solution was determined by addition of 50µl of the standard solution to 150µl calf plasma.

Additional Information		
	Part 4	Part 5
Sample pretreatment	1) Add 1 mL of 0.5 M tetrabutylammonium bisulfate solution (adjusted to pH 10 with 10 M and 1 M NaOH) 2) Add 2 mL of carbonate/ bicarbonate buffer solution	None
Extraction technique:	Liquid-liquid extraction MTBE	SPE using Waters Oasis cartridges
Extraction solvents:	Final extract contains 20% ACN in HPLC-grade water (1mL extract) Discovery HS C18 7.5, cm x 2.1mm, 3 µm	ACN followed by SPE where MeOH was used to elute the compounds of intrerest
Clean Up:	Discovery HS C18, 5 cm x 2.1mm, 5 µm and Acentis C18, 5 cm x 2.1 mm x 5 µm Discovery C18, 10 m x 2.1 mm, 3 µm Acetonitrile: 2 mM ammonium acetate (20:80)	None
LC column:	Acetonitrile: 2 mM ammonium acetate (90:10) 200 µL/min 10 µL	Betasil C-18, 100 x 2.1, 5um MeOH and 2 mMolar Ammonium Acetate
LC/MS(MS):	The gradient elution started with 100% A for 1 min, followed by an 8 min linear gradient to 100% B, then 5 min hold at 100% B, and returned back to 100% A in 4 min. The system was equilibrated for 4 min at the initial conditions before the next injection.	SCIEX API 3000, MS/MS, ESI
(Labelled) Internal Standards: (Labelled) Recovery Standards: Results Corrected for Recovery: Results Corrected for Blanks:	Finnigan Surveyor Plus LC System (Thermo Electron Corporation) TSQ Quantum UltraTrace MS/MS (Thermo Electron Corporation) Negative ESI mode using Selective Reaction Monitoring	MPFAC-MXA-100 MPFAC-MXA-100 No No
Standard Method:	8 labelled internal standards, refer to MPFAC-MX-100 for internal standard list Results corrected for blank using an extracted calibration curve with sterlie goat serum	Reagen et al. Analytica Chemica Acta (2008)
Comments:	N/A	A gaurd column was used to elminate background contamination coming from the eluents or HPLC It was a Prism RP 50 x 2.1, 5um

Additional Information			
	Part 6	Part 7	Part 8
Sample pretreatment	Thaw same day.	Alkaline digestion	Additon of 1.0 mL of 1.0 N Formic Acid, 100 uL of saturated ammonium sulfate, vortex sample
Extraction technique:	Protein precipitation. Add 200 uL of sample to a 1.5 mL centrifuge tube. Add 25 uL of spiking solution. Add to MultiPROBE and mix sample three times by aspirating and dispensing 100 uL of sample.		Solid Phase Extraction (SPE) Water Oasis HLB cartridges
Extraction solvents:	Add 775 uL of acetonitrile.	MeOH/KOH	NA
Clean Up:	Centrifuge sample at 10,000 rpm for 20 minutes. Transfer supernatent to an autovial with 60 uL of a 5% phosphoric acid solution.	SPE HLB and SPE Envi Carb	NA
LC column:	Prism RP 2 x 50 mm, 5 um Guard Column Prism RP 2 x 50 mm, 5 um Mobile Phase: A-5 mM Ammonium Ac Extraction Column: Oasis HLB Online column, 3 x 20, 25 um Analytical Column: Betasil C18 2.1 x 100 mm, 5 um Guard pre-autosampler: Prism RP 2 x 50 mm, 5 um For PFBS, PFHS, PFOS, FOSA, 18O2 PFBS, MPFOS, and 18O2 PFOS Extraction Column: Oasis HLB Online column, 3 x 20, 25 um Analytical Column: Betasil C18 2.1 x 100 mm, 5 um Guard Colomn	C18 3µm (50 x 2,0 mm) A : MeOH ; B : H2O/AcONH4 0.02M	Mac-Mod ACE C-18, 5 micron 75mm x 2.1 mmi.d. Base Deactivated Column
LC/MS(MS):	Applied Biosystems MDS/Sciex API 5000 (MS/MS)	LC-MS/MS Agilent QqQ 6410 ESI	Applied Biosystems / Sciex API 5000
(Labelled) Internal Standards:	[1,2,3,4-13C4]PFBA	8	13C2 PFOA, 18O2 PFOS, 18O2 PFBS, 18O3 PFHxS, 13C4 [1,2,3,4-13C4] PFBA
(Labelled) Recovery Standards:	[1,2-13C4]PFHxA	1	
Results Corrected for Recovery:	[1,2-13C4]PFHxA	Yes	no
Results Corrected for Blanks:	[1,2-13C4]PFHxA	Yes	no
Standard Method:	[1,2,3,4-13C4]PFOA [1,2,3,4-13C4]PFNA [1,2-13C2]PFDA	Internal method	
Comments:	[1,2-13C2]PFUnA [1,2-13C2]PFDoA [18O2]PFBS [18O2]PFHS [1,2,3,4-13C4]PFOS [18O2]FOSA		

Additional Information		
	Part 9	Part 10
Sample pretreatment	4 ml of 0.25 M sodium carbonate (for liquid liquid extraction)	
Extraction technique:	0.3 g of sample extracted in duplicate or triplicate using 1 ml 0.5 M TBAS ion pairing agent (pH 10)	LL
Extraction solvents:	5 ml x 2 methyl tert butyl ether	0.05 N KOH/MeOH
Clean Up:	none	SPE; STRATA X-AW
LC column:	Luna C8, 50 x 2 mm, 3 micron (Phenomenex, Torrance, CA, USA, part number 00B-4248-B0) for PFHxS, PFOA to PFDoA, and PFOS Shodex Rspak JJ-50 2D anion exchange/reverse phase column, 15 x 2 mm, 5 um (part number J803002, Shodex, Shanghai) METHANOL/WATER(AMMONIUM ACETATE BUFFER) gradient was used	Synergy 4u Fusion RP C-18 (100 mm × 2.0 mm i.d.)
LC/MS(MS):	Agilent 1100 pump and AB MDS Sciex 4000 QTRAP (MS/MS)	Varian Model 1200; LC-MS/MS-ESI
(Labelled) Internal Standards:	C13 PFBA, PFHxA, PFOA, PFNA, PFDA, PFUnA, PFDoA, and PFOS, and 18O PFHxS all from Wellington	7
(Labelled) Recovery Standards:	recovery was conducted using native standards spiked into serum.	1
Results Corrected for Recovery:	no see comment	No
Results Corrected for Blanks:	no see comment	Yes
Standard Method:	no	inhouse method
Comments:	Did not correct for blanks. Triplicate method blanks were clean with the exception of PFOA (0.05 ng/ml) and PFOS (0.004 ng/ml) Did not correct for recovery; however, recovery corresponded to 120% for PFOA, 90% PFNA, 100% PFDA, 110% PFUnA, 95% PFDoA, 95% PFTrA, 99% PFOS and 97% PFHxS in serum.	

Additional Information		
	Part 11	Part 12
Sample pretreatment	dilution with 0.1M formic acid	No pretreatment
Extraction technique:	Oasis HLB SPE, 60 mg, 3cc cartridges	Liquid-liquid extraction
Extraction solvents:	1 mL 1% NH4OH in ACN	Methyl tert-butyl ether, 0.25 M sodiumcarbonate, 0.5 M tetrabutylammoniumhydrogensulphate (pH 10)
Clean Up:	none	No additional clean up, except centrifugation
LC column:	Betasil, C8 3um, 2.1 x 5 mm 0.1% formic acid in H2O and 0.1% formic acid in ACN at 400 uL/min, 30%B, 0.25 min. Ramp to 90%B over 2 min. Hold @90%B till 7 min.	C18 125*2 mm 2 mM ammoniumacetate in water and 2 mM ammoniumacetate in methanol
LC/MS(MS):	Applied Biosystems 4000 Qtrap ESI	TSQ Thermo ESI
(Labelled) Internal Standards:	MPFBA, MPFHxA, MPFOA, MPFNA, MPFDA, MFPHxS, MFPOS	2
(Labelled) Recovery Standards:		1
Results Corrected for Recovery:	no	No
Results Corrected for Blanks:	no	Yes
Standard Method:		
Comments:	Our method was developed and validated for serum using matrix matched calibration curve (bovine calf serum). We would have liked to extract a 1 ml aliquot of sample (as this is how we developed and validated our method). There was only 1 mL of sample and we typically do duplicates or matrix spikes for samples, we instead extracted 250uL of sample.	

Additional Information		
	Part 13	Part 14
Sample pretreatment	Samples frozen on receipt, and thawed just prior to PFC analysis. Plasma sample were transferred into a polypropylene tube and spiked with MPFAC-MX-100 and d-N-Me-FOSA Standard MX3 was spiked with MPFAC-MX-100 in HPLC vial for HPL	1. 100 mL of plasma was measured into 750 mL polypropylene autosampler vial 2. 25 mL internal standard and 275 mL 0.1 M formic acid was added
Extraction technique:	Extracted by homogenizing the sample for 1 min with the homogenizer. Centrifuged for 10 min at 5000 rpm to separate the extract. Transfer the extract to a tube. Repeated the extraction process 2 more times and combine the extracts in the same tube.Conce	3. The vial was closed with a polypropylene snap cap and vortex mixed for 5 seconds. Solid phase extraction-Symbiosis (Spark Holland) on-line solid phase extraction system 10x1 mm Polaris C18 Prospect-2 cartridge (Varian)
Extraction solvents:	10 mM KOH acetonitrile/water (80V/20V) solution	1. Condition a SPE cartidge with 2 mL acetonitrile and 2 mL 0.1 M formic acid 1. 400 µL of the sample (containing 100 µL serum) is loaded onto the SPE column using 3 mL of 0.1 M formic acid with 1 mL/min flow rate
Clean Up:	Oasis WAX SPE cartridge, 3 mL, 60 mg, 30 um	2. 1 mL of 50% 0.1 M formic acid/50% methanol 3. 300 µL of 0.3% NH4OH/water
LC column:	C-18 50 mm X 0.21 mm i.d., 3 um A: 2mM ammonium acetate in water; B: 2mM ammounium acetate in methanol 0-10 min from 95% A to 20 % A; 10-20 min from 20 % A to 0 % A and hold for 5 min.	HPLC mobile phase HPLC column: Betasil C8, 50x3 mm, 5 µm, Thermo Electron Corporation, Bellefonte, PA HPLC pre-column: Betasil C8 precolumn 3 × 10 mm, 5µm Flow rate : 600 microL/ min
LC/MS(MS):	MS/MS with ESI for PFAs and PFASs MS/MS with APPI for PFOSA using water and methanol as mobile phases	Mobile phase A : 20 mM ammonium acetate, pH 4 Mobile phase B : methanol, HPLC grade, Gradient
(Labelled) Internal Standards: (Labelled) Recovery Standards: Results Corrected for Recovery: Results Corrected for Blanks:	10 No No	 API 4000 tandem mass spectrometer in the negative ion Turbo Ion Spray (TIS) mode
Standard Method:		
Comments:	The concentration of PFBS is in potasium salt form. The concentrations of PFHxS, PFOS and PFDS are in sodium salt form. LOD = 0.1ng/ml	6 (13C2-PFOA, 18O2-PFOS, 13C5-PFNA, 13C2-PFDeA, 13C2-PFDoA, and 18O2-PFHxS) None No No N/A

We did not use MPFAC-MX-100, but our own internat standard mixture
1. 100 mL of calf serum was measured into 750 mL polypropylene autosampler vial
2. 25 mL standard MX3, 25 mL internal standard, and 250 mL 0.1 M formic acid was added
3. The vial was closed with a polypropylene snap cap and vortex mixed for 5 seconds.
4. Same SPE and HPLC conditions as those used for the plasma samples

Additional Information				
	Part 15	Part 16	Part 17	Part 18
Sample pretreatment	None	TBA solution	TBA	NA NA
Extraction technique:	Liquid/Liquid Extraction	LL	LL	NA NA
Extraction solvents:	ACN	MTBE	MTBE	NA NA NA
Clean Up:	SPE (Oasis®WAX, 150mg, Waters)	filtration	filtration	NA NA NA
LC column:	Keystone Betasil C 18 Column 2.1 mm I.D. × 50 mm length, 5µm, 100 Å pore size 2 mM ammonium acetate: MeOH	C18 1.7um*50mm	C18 2.1*50mm	NA NA NA NA
LC/MS(MS):	MS/MS, Waters ESI	UPLC-MS/MS ESI	UPLC-MS/MS ESI	NA NA NA NA
(Labelled) Internal Standards:	MPFAC-MXA-100	MPFAC-MXA-100	MPFAC-MXA-100	NA
(Labelled) Recovery Standards:	MPFAC-MXA-100			NA
Results Corrected for Recovery:	Never !! We strongly disagree with data correction using RS.	Yes	Yes	NA
Results Corrected for Blanks:	Never !! We strongly disagree with data correction using RS.	Yes	Yes	NA
Standard Method:	ISO 25101 Water quality — Determination of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) — Method for unfiltered samples using solid phase extraction and liquid chromatography/mass spectrometry (March 1, 2009) Except ACN liquid liquid extraction			 NA NA
Comments:	For PFPeA in serum samples, considerable amount was measured in betasil column. The retention time of the peak for PFPeA in the sample is same as the peak of native standard. However, when using different type of column to confirm, PFPeA cannot be detected. Therefore, it was thought that the peak detected in Betasil column may not be PFPeA. Hence, we didn't report PFPeA for serum samples.			

Additional Information		
	Part 19	Part 20
Sample pretreatment	none	-
Extraction technique:	protein precipitation with ACN	solvent shaking
Extraction solvents:	ACN	MeOH
Clean Up:	none only dilution with water	activated charcoal
LC column:	Halo C8 50x2.1mm; 2.7µm (fused core particle) Flow: 400 µl/min A: 2mM NH4acetate B: MeOH 0 min: 10%B - 0.75min:40%B - 6min: 95%B than back to start conditions (3.5min)	Atlantis T3 (100 mm x 4 mm i.d.; 5 µm) gradient MeOH - 2mM ammonium acetate
LC/MS(MS):	MS/MS (MRM mode) API4000 (Applied Biosystems) analysed in ESI neg (Turbolon Spray)	Alliance 2695/Quattro Premier XE, Waters ESI- MS/MS (triple quadrupole)
(Labelled) Internal Standards: (Labelled) Recovery Standards: Results Corrected for Recovery: Results Corrected for Blanks:	mixture received with samples (Wellington labs) (13C2-PFOA) yes yes	yes - 2 (Perfluoro-n-[1,2,3,4-13C4]octanoic acid-PFOA; Sodium perfluoro-1-[1,2,3,4-13C4]octasulfonate - PFOS) No No
Standard Method:		In house validated
Comments:		In addition to them we have detected following compounds (in brackets only semiquantitative results are given - ng/ml): PFHxA (0.7); PFHxS (3); PFHpA (0.5); PFNA (1.3); std MX3 (ng/ml): PFBS (176); PFHxA (171); PFHxS (144); PFHpA (128); PFNA (187); PFDA (153); PFUdA (126); PFDoA (86)

Additional Information		
	Part 21	Part 22
Sample pretreatment	N.a.	
Extraction technique:	LLE	vortex, sonication
Extraction solvents:	MeOH	acetonitrile
Clean Up:	Envicarb	dispersive carbon (ENVI-Carb)
LC column:	Symmetry C18 2.1x50 mm 5 µm, Waters	Acquity BEH C18 50*2.1mm, 1.7um
LC/MS(MS):	Agilent 6410 ESI-QQQMS and Agilent 1200 LC system time (min) MeOH 2mM ammoniumacetate flow: 0.3 ml/min 0 10 90	Acquity UPLC Quattro Premier XE MS/MS
(Labelled) Internal Standards:	0.5 10 90	C6,C8 sulfonates, C4,C5,C8,C9,C10;c11,C12 carboxylates
(Labelled) Recovery Standards:	50 100 0	7H-PFHpA
Results Corrected for Recovery:	50.5 100 0	yes
Results Corrected for Blanks:	50.6 10 90	no (blanks insignificant)
Standard Method:	65 10 90 9 Mass labelled internal standards (13C-PFBA, 13C-PFHxA, 13C-PFOA, 13C-PFNA, 13C-PFDA, 13C-PFUnA,18O-PFHxS, 13C-PFOS, 18O-PFOSA) None	
Comments:	Yes (by using labeled standards) Yes N.a. Everything quantified based on a calibration curve made up in solvent (so no matrix matched). We used the Wellington linear isomer standards and quantified all isomers based on this standard (so, we neglected response factor differences)	

We used our own mass labeled IS solution. All our IS come from Wellington, except 18O-PFOSA.
We checked our solution against the provided mix and there was reasonable agreement (max deviation 6%) except for PFHxS which showed 15% difference. The instrumental variance (between different injections) can play a role here and it was not possible to account for this.

Please note that one of the serum samples had a somewhat loose cap when we unpacked it.
The were no signs of sample leakage, but some evaporation of moist may have occurred possibly.