Quantitative Determination of PFOS, PFOSA, PFOSAA, POAA, PFHS, M556 and M570 in Human Serum by LC/MS/MS

Assay Revalidation Addendum Report

Northwest Bioanalytical (NWB)
A Division of NWT Inc.
1121 East 3900 South
Salt Lake City, UT 84124

PREPARED FOR: 3M Environmental Technology and Services
935 Bush Avenue
St. Paul, MN 55133

AUTHOR:
Connie O Sakashita DATE: 11-20-01
Connie O. Sakashita, B.S., NWB Project Manager

APPROVED FOR RELEASE BY:
Patrick Bennett DATE: 11-20-01
Patrick Bennett, M.S., M.B.A., NWB Laboratory Director

QUALITY ASSURANCE STATEMENT

LABORATORY:	Northwest Bioanalytical (NWB) A Division of NWT, Inc. 1121 East 3900 South Salt Lake City, UT 84124
SPONSOR:	3M Environmental Technology Services 935 Bush Avenue St Paul, MN 55133
COMPOUND(S):	PFOS, PFOSA, PFOSAA, PFHS, M556 and M570
NWB STUDY NUMBER:	NWBS00-040
SPONSOR STUDY NUMBER:	Method Validation
NWB STUDY TITLE:	Quantitative Determination of PFOS and Metabolites in Human Serum by LC/MS/MS, Assay Validation

The method validation study described in this report is not included within the definition of a GLP regulated nonclinical study. However, Northwest Bioanalytical conducts all projects in accordance with the guidelines of the U.S. FDA Good Laboratory Practice Regulations for Nonclinical Laboratory Studies (Title 21 CFR Part 58), the OECD Principles of Good Laboratory Practice and the Japanese MHW Good Laboratory Practice Standard Ordinance for Nonclinical Laboratory Studies on the Safety of Drugs (Ordinance No. 21, PAB Notification No. 424.) The following inspections were performed by the NWB QAU per SOP.

Inspection and Reporting Statement.

		Date Inspection Report Issued To	
Inspection Date	Phase of Study	NWB Project Manager	[1]NWB Management
15 May 2000	Analytical Plan	15 May 2000	31 May 2000
15-18 May 2000	Assay Validation	18 May 2000	31 May 2000
07-18 Sep 2000	Report Draft/Raw Data (NWBR00-108)	20 Sep 2000	29 Sep 2000
24 Jan 2000	Final Report (NWBR00-108)	24 Jan 2000	31 Jan 2000
19-30 Jul 2001	Report Draft/Raw Data (NWBR00-122)	30 Jul 2001	31 Jul 2001
05-07 Nov 2001	Report Draft/Raw Data (NWBR00-122)	08 Nov 2001	30 Nov 2001
20 Nov 2001	Final Report (NWBR00-122)	20 Nov 2001	30 Nov 2001

[1]Reports to NWB Management are issued monthly.

As can reasonably be established, the methods and procedures described and the results incorporated into this final report accurately reflect the raw data.

Sheryl A. Mathews

Sheryl A. Mathews, A.A.S.
NWB QAU Compliance Auditor

20 Nov 2001

Date

COMPLIANCE STATEMENT

The method validation study described in this addendum report is not included within the definition of a GLP nonclinical regulated study. However, to the best of our knowledge, the stability, extraction efficiency, and plasma abbreviated validation experiments were conducted in accordance with the guidelines of the U.S. FDA Good Laboratory Practice Regulations for Nonclinical Laboratory Studies (Title 21 CFR Part 48) and according to the methods and procedures described within this report. In addition, these experiments followed the guidelines of the OECD Principles of Good Laboratory Practice and the Japanese MHW Good Laboratory Practice Standard Ordinance for Nonclinical Laboratory Studies on the Safety of Drugs (Ordinance No. 21, PAB Notification No. 424.)

This addendum report also included results from research experiments conducted outside of the validation study. These experiments were conducted according to the procedures described within section 7 of this report.

Serum sample results obtained using plasma curves for PFOSA, PFOSAA, POAA, PFHS, M556 and M570 did not meet NWB SOP requirements for validation.

Any known circumstances that may have affected the quality or integrity of the study or data are discussed within the report. This report represents an accurate record of the raw data.

Connie O Sakashita Date: 11-20-01

Connie O. Sakashita, B.S.
NWB Project Manager

Patrick K. Bennett Date: 11-26-01

Patrick K. Bennett, M.S., M.B.A.
NWB Laboratory Director

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1. INTRODUCTION

Northwest Bioanalytical (NWB) was contracted by 3M Environmental Technology and Services (3M) to modify and revalidate a liquid chromatography/tandem mass spectrometry method for the measurement of PFOS, PFOSA, PFOSAA, POAA, PFHS, M556 and M570 in human serum. The accuracy and precision results of the method revalidation were reported in NWB report NWBR00-108 [9.1]. This report summarizes the results from the stability tests conducted as part of the method revalidation for PFOS, PFOSA, PFOSAA, POAA, PFHS, M556 and M570 in human serum for 3M Environmental Technology and Services. The results from the following tests are also included: extraction efficiency from human serum; ion suppression experiment; and diluted matrix experiment. The suitability of the method for use with plasma was determined by demonstrating intra-assay and LLOQ accuracy and precision and comparing serum and plasma results.

Kris Hansen and James Lundberg at 3M Environmental Technology and Services served as the Study Monitors. The following is a list of NWB supervisory personnel involved in the completion of this work: Connie O. Sakashita, B.S. (Current NWB Project Manager); Brad I. Coopersmith, Ph.D. (NWB Project Manager until October 28, 2000); Licong Jiang, Ph.D. (NWB Senior Scientist); Patrick Bennett, M.S., M.B.A. (NWB Laboratory Director). The following NWB laboratory personnel contributed to the completion of this work: Suzanne Newman, B.S. (NWB Scientist).

NWB SOPs were used in the conduct of this project and were available to project personnel in both electronic and hard copy formats.

Date Study Initiated: 13-Apr-2000 Date Analyses Completed: 25-Apr-2001

The method revalidation study described in this report is not included within the definition of a GLP regulated nonclinical study. However, Northwest Bioanalytical conducts all studies within the guidelines of the US FDA Good Laboratory Practice Regulations for Nonclinical Laboratory Studies (Title 21 CFR Part 58), the OECD Principles of Good Laboratory Practice and the Japanese MHW Good Laboratory Practice Standard Ordinance for Nonclinical Laboratory Studies on the Safety of Drugs (Ordinance No. 21, PAB Notification No. 424). Any changes to or deviations from the original protocol (Analytical Plan) were documented through approved protocol amendments or deviation memos and are retained within the raw data.

2. METHODOLOGY

Reference Materials and Matrices

Analyte	*Lot Number*	*Purity*	*Expiration Date*	*Source*	*Storage Conditions*
PFOS (FC-95)	193	100%	12/31/2010	3M	Room Temperature
PFOSA	214	100%	12/31/2010	3M	Room Temperature
PFOSAA (FC-129)	617	53.8%	12/31/2010	3M	Room Temperature
POAA (FC-143)	245	100%	12/31/2010	3M	Room Temperature**
PFHS* (used for serum)	S398-182	100%	12/31/2010	3M	-20°C
PFHS (used for plasma)	SE-036	91.1%	1/01/2010	3M	-20°C
M556	NB113047-8D	99.89%	12/31/2010	3M	Room Temperature
M570	118506-26	99.75%	12/31/2010	3M	Room Temperature
THPFOS	59909	90%	12/31/2010	3M	Room Temperature

* Received as a 6,200 ppm solution in methanol. ** Kept dry.

Matrix	*Lot Numbers*
Human serum	BC30399-4; BC061900-1
Human plasma (Northern Chinese)	C5186; C4929; C5517; C5200

The reference material purity for PFOS, PFOSA, PFHS and POAA was not available prior to the conduct of this study. Therefore, all serum concentrations included in the report for these analytes are based upon an assumed purity of 100%.

After the revalidation was completed, 3M contracted with Centre Analytical Laboratories, Inc. in State College, Pennsylvania to determine the absolute concentration of PFOS, POAA and PFHS in NWB stock solutions used to prepare the analytical standards and controls used for this revalidation and subsequent analyses. All arrangements for purity determinations and transfers of NWB solutions to Centre Analytical Laboratories, Inc. were performed by 3M. Per agreement with the Sponsor, NWB quantitative results were not corrected for the purity corrections. Therefore, based on the results obtained, the serum concentrations included in this report should be corrected according to the following table (except for the serum samples included in the serum versus plasma experiments, Section 7.3):

Analyte	Correction Factor
PFOS	0.836
POAA	0.909
PFHS	0.855

Principles of the Method

The analytical method consisted of a liquid:liquid extraction procedure followed by evaporation and reconstitution of the extract residue with 30:70 (v/v) 20 mM ammonium acetate in water: 20 mM ammonium acetate in methanol. The samples were analyzed by liquid chromatography/tandem mass spectrometry using a PE Sciex API 3000. The instrument was operated in the multiple reaction monitoring (MRM) mode under

optimized conditions for PFOS, PFOSA, PFOSAA, POAA, PFHS, M556 and M570 detection.

A detailed description of the methodology is included in NWB report NWBR00-108 [9.1].

Acceptance Criteria for Human Serum

Calibration Curve

Each run will include in duplicate calibration standards at six or more concentrations covering the lower to upper limit of quantitation. For all analytes except PFOSA, at least three-fourths of the calibration standard's back-calculated concentrations must be within ± 15% (± 20% for LLOQ) of their individual target concentrations. For PFOSA, at least three-fourths of the calibration standard's back-calculated concentrations must be within ± 20% (± 25% for LLOQ) of their individual target concentrations. A calibration standard will be considered a statistical outlier if the back-calculated concentration is greater than two times the acceptance criteria for that standard.

Lower Limit of Quantitation

The back-calculated concentrations of at least one of the duplicate lowest points in the calibration curve must be within ± 25% of the target concentration for PFOSA to qualify as the LLOQ and within ± 20% of the target concentration to qualify as the LLOQ for all other analytes. If this criterion is not met, the next level is subjected to the same test and the LLOQ raised accordingly.

Quality Control Samples

Each analytical run will include low, medium and high QC samples in duplicate. The measured concentrations of at least two-thirds of all analytical QCs must be within ± 20% of their target concentrations (± 25% for PFOSA), and no two QCs at the same concentration can be outside the limit. If study samples require dilution, a dilution QC will be analyzed in triplicate for each dilution level (except for PFOSA and PFOSAA

which should not be diluted with control matrix). At least two dilution QC at each level must be within ± 20% of its target concentrations in order to accept diluted study samples at that level. The dilution QC acceptance is independent of the undiluted analytical QC acceptance.

3. STABILITY EVALUATION

The revalidated method's final acceptance criteria is used as a basis for evaluating fluorochemical stability results. If the mean concentration obtained for stability test QC samples falls within ± 20% (± 25% for PFOSA) of the mean control QC concentrations, then stability is considered acceptable under the specified test conditions. Control QC samples are defined as samples not subjected to test conditions.

The calibration curve parameters summary and individual calibration standard results for the serum stability runs can be found in Tables 1-14. The analytical QC results for the stability runs can be found in Tables 15-21.

3.1. Freeze-Thaw and Room Temperature Matrix Stability

The stability of PFOS, PFOSA, PFOSAA, POAA, and PFHS in human serum for three freeze-thaw cycles was demonstrated and reported under the original validation study NWBS98-092, NWB report NWBR99-005 [9.2]. Therefore, only freeze-thaw stability for M556 and M570 was needed. Acceptable results for the three freeze-thaw cycle stability test was obtained on Run 4 for M556 and M570 (data not included).

However, since some analyzed subject samples subsequently underwent more than three freeze-thaw cycles, the stability of PFOS, PFOSA, PFOSAA, POAA, PFHS, M556 and M570 was evaluated after seven freeze-thaw cycles. All analytes demonstrated acceptable stability when compared against controls that underwent one freeze-thaw cycle (Tables 22 – 28).

During the seven freeze-thaw cycle test, samples were left at room temperature for up to 6.75 hour periods between each freeze cycle. Since the freeze-thaw samples

demonstrated acceptable stability, the stability of serum samples left at room temperature for up to 6.75 hours is also demonstrated.

3.2. Reinjection Stability

The quantitative reproducibility of standards and controls after reinjection was determined for PFOS, PFOSA, PFOSAA, POAA, PFHS, M556, and M570. The samples were extracted and analyzed on June 30, 2000 (Run 15 – data shown in NWBR00-108). These samples were reinjected on July 3, 2000 after storage at room temperature (Run 23). The results from Run 23 indicate reinjection stability when compared with data obtained from the original injection [Run 15] (Tables 29 – 35).

3.3. Extract Storage Stability

Extract stability was evaluated at different storage temperatures and for various lengths of time. The evaluations were performed using QC lots prepared on June 27, 2000 for all analytes and conditions. Although the same QC lots (prepared on June 27, 2000) were not used for PFOSA and PFOSAA run acceptance determination, they still provided a control value for stability evaluations.

3.3.1. Room Temperature Extract Stability

The extract stability of PFOS, PFOSA, PFOSAA, POAA, PFHS, M556 and M570 was determined for low and high serum QC extracts stored at room temperature for seven days prior to analysis. These test QC samples were compared against serum control samples that were analyzed immediately following extraction.

The extracted test QC samples demonstrated acceptable stability after seven days at normal room temperature (Tables 36 – 42).

3.3.2. Reduced Temperature (1 to 8°C) Extract Stability

The extract stability of PFOS, PFOSA, PFOSAA, POAA, PFHS, M556 and M570 was determined for low and high serum QC extracts stored at 1 to 8°C for four

days prior to analysis. These test QC samples were compared against serum control samples analyzed immediately following extraction.

The extracted test QC samples demonstrated acceptable stability after four days at 1 to 8 °C (Tables 43 – 49).

3.3.3. Reduced Temperature (-20 °C) Extract Stability

The extract stability of PFOS, PFOSA, PFOSAA, POAA, PFHS, M556, and M570 was determined for low and high serum QC extracts stored at -20 °C for five days prior to analysis. These test QC samples were compared against serum control samples analyzed immediately following extraction.

The extracted test QC samples demonstrated acceptable stability after five days at -20 °C (Tables 50 – 56).

3.4. Long-Term Matrix Stability in Human Serum

The long-term matrix stability of PFOS, POAA, PFHS, M556 and M570 in human serum was determined by storing QC samples for approximately 55 days at –20 °C. The long-term matrix stability of PFOSA and PFOSAA in human serum was determined by storing QC samples for approximately 42 days at –20 °C. The mean test QC concentrations were compared against theoretical.

The test QC samples demonstrated acceptable stability after approximately 42 days for PFOSA & PFOSAA or 55 days for other analytes, at –20 °C (Tables 57 – 63).

4. EXTRACTION EFFICIENCY FROM HUMAN SERUM

The extraction efficiencies of PFOS, PFOSA, PFOSAA, POAA, PFHS, M556 and M570 were determined by comparing the area ratios obtained for the following three cases:

1. Both the analyte and internal standard added following the extraction (post-extract).

2. The analyte added to serum prior to extraction and the internal standard added following extraction (pre-extract analyte).
3. The internal standard added to serum prior to extraction and the analyte added following the extraction (pre-extract internal standard).

The extraction efficiencies were then determined by the area ratio of the pre-extract samples to the post-extract samples. The extraction efficiency experiments were performed at both low and high concentrations to determine that there was no concentration bias.

The total amount of analyte in the sample prior to extraction (pre-extract) is equal to the amount of analyte added plus the persistent amount in the blank matrix. The amount of analyte in the post-extract solution is the amount of analyte added plus the amount of analyte recovered (extracted) from the blank matrix. Therefore, the following formula was used to determine the extraction efficiency for PFOS, PFOSAA, POAA, PFHS, M556 and M570:

$$((Spk + Per)^* EE) / (Spk + (Per * EE)) = Rpre / Rpost; \text{ where}$$

Spk = the amount of analyte added
Per = the persistent amount of analyte in the blank matrix
EE = the extraction efficiency
Rpre = the ratio of analyte/internal standard determined for the analyte added prior and internal standard added after extraction
Rpost = the ratio of analyte/internal standard determined for the analyte and internal standard added after extraction

	PFOS	PFOSA	PFOSAA
Mean extraction efficiency (%)	37.3	69.6	62.7

	POAA	PFHS	M556
Mean extraction efficiency (%)	15.6	15.3	39.1

	M570	THPFOS
Mean extraction efficiency (%)	57.1	5.99

The results are shown in Tables 64 – 70.

5. COMMENTS AND CONCLUSIONS

Per agreement with the Sponsor, the regressions were not recalculated based upon the updated purity information for PFOS, POAA and PFHS. Some differences might occur due to the effect of rounding if the regressions were performed with the purity corrected concentrations, but these differences would have a negligible effect on the overall interpretation of the revalidation results.

PFOS, PFOSA, PFOSAA, POAA, PFHS, M556 and M570 in human serum appear to be stable for seven freeze-thaw cycles and demonstrated matrix stability for up to 6.75 hours at room temperature. PFOS, PFOSA, PFOSAA, POAA, PFHS, M556 and M570 exhibit extract stability for seven days at room temperature, four days at 1 to 8 °C, and five days at -20 °C. The analytes also demonstrated matrix stability for approximately 55 days (for PFOS, POAA, PFHS, M556 and M570) and 42 days (for PFOSA and PFOSAA) at -20 °C in human serum.

6. ABBREVIATED VALIDATION – HUMAN PLASMA

The choice of matrix for calibration curve preparation is dependent upon the desired LLOQ. 3M supplied NWB with human plasma identified as "Northern Chinese" plasma (referred to as Chinese plasma or CP). This matrix has very low levels of fluorochemicals and will be used for studies with a desired LLOQ of ≤ 5.00 ppb. The following sections summarize the accuracy and precision of the fluorochemicals in human plasma and the quantitative accuracy of serum sample results obtained against plasma calibration curves using the revalidated serum method.

The absolute concentrations of PFOS, PFHS and POAA in the stock solution used for plasma analyses were determined by Centre Analytical Laboratories, Inc. in State College, Pennsylvania. Based upon the results, the concentrations of plasma standard and quality control samples were corrected using the following factors:

Analyte	Correction Factor
PFOS	0.821
POAA	0.961
PFHS	1.043

All plasma experiment concentrations included in this report reflect adjustments based upon the correction factors above. The serum target concentrations for these experiments have also been adjusted based upon the correction factors (listed on page 15 of report).

The tests for the abbreviated human plasma validation were conducted after the serum revalidation was completed. They were performed outside of the revalidation or in sample analysis studies conducted after the serum revalidation. However, all applicable NWB SOPs were followed.

Persistent Levels of Analytes in Chinese Plasma

The persistent levels of the analytes in Chinese plasma varied in each tube of plasma tested. Therefore, several tubes of plasma were pooled and the amounts of persistent analytes were determined. Previous determination of persistent levels was achieved by comparison with rabbit serum curves. For the experiments included in this report, the persistent levels were determined as follows:

Known amounts of the analytes were added to Chinese plasma in the range of 1 to 50 or 1 to 100 ppb. The standards were then analyzed according to the revalidated method on at least two separate occasions. A linear regression was performed using the spiked analyte concentration as the y-value and the ratio of the analyte area to internal standard area as the x-value (Microsoft® Excel 97 formulas). The mean resultant y-intercept determined from multiple runs was then used as an estimate of the persistent level of analyte if it was greater than 0.2 ppb.

6.1. Range of Quantitation

The target calibrator concentrations were approximately 1.00 , 2.50, 10.0, 25.0, 50.0, 100, 250, 400 and 500 ppb for all analytes except M556. The target calibrator concentrations for M556 were approximately 2.50, 10.0, 25.0, 50.0, 100, 250, 400 and 500 ppb. The final curve range can vary based upon the persistent levels of the analyte in the human plasma pool used. For the validation study, the calibration curve ranges were as follows:

PFOS	3.94 to 414 ppb
PFOSA	1.00 to 500 ppb
PFOSAA	1.60 to 501 ppb
POAA	1.92 to 481 ppb
PFHS	1.36 to 523 ppb
M556	2.50 to 500 ppb
M570	1.00 to 500 ppb

The calibration curve parameter summaries and individual calibration standard results for the plasma runs can be found in Tables 71 – 84.

6.2. Lower Limit of Quantitation

The precision at the Lower Limit of Quantitation (LLOQ) of the assay in human plasma was determined by using six 100-µL aliquots of the pooled Chinese plasma fortified with PFOS, PFOSA, PFOSAA, POAA, PFHS, M556, and M570 at concentrations equal to the LLOQ. The LLOQ concentration was dependent upon the persistent levels found in the plasma.

	PFOS	PFOSA	PFOSAA	POAA
Lower Limit of Quantitation	3.94 ppb	1.00 ppb	1.60 ppb	1.92 ppb
Precision (%CV)	5.4	10.1	5.7	4.4
Accuracy (%Bias)	-1.5	1.0	-2.5	-8.9

	PFHS	M556	M570
Lower Limit of Quantitation	1.36 ppb	2.50 ppb	1.00 ppb
Precision (%CV)	9.9	5.7	5.2
Accuracy (%Bias)	-5.1	-2.0	3.0

The results are shown in Tables 85 – 91.

6.3. Intra-Assay Precision and Accuracy

The precision and accuracy of the LC/MS/MS method for PFOS, PFOSA, PFOSAA, POAA, PFHS, M556, and M570 in human plasma were determined by analyzing three levels of quality control samples in replicates of five on a single run.

	PFOS Intra-assay	PFOSA Intra-assay	PFOSAA Intra-assay	POAA Intra-assay
Precision (%CV)	1.5 to 2.8	1.8 to 5.7	2.6 to 5.8	0.8 to 5.8
Accuracy (%Theoretical)	101.2 to 112.3	104.0 to 116.3	102.6 to 111.7	103.4 to 106.2
Accuracy (%Bias)	1.2 to 12.3	4.0 to 16.3	2.6 to 11.7	3.4 to 6.2

	PFHS Intra-assay	M556 Intra-assay	M570 Intra-assay
Precision (%CV)	1.7 to 3.1	4.7 to 7.6	1.9 to 5.6
Accuracy (%Theoretical)	95.7 to 106.0	98.7 to 111.3	101.3 to 118.0
Accuracy (%Bias)	-4.3 to 6.0	-1.3 to 11.3	1.3 to 18.0

The results are shown in Tables 92 – 98.

6.4. Proposed Acceptance Criteria for Human Plasma

Calibration Curve

Each run will include in duplicate calibration standards at six or more concentrations covering the lower to upper limit of quantitation. For all analytes except PFOSA and PFOSAA, at least three-fourths of the calibration standard's back-calculated concentrations must be within ± 15% (± 20% for LLOQ) of their individual target

concentrations. For PFOSA and PFOSAA, at least three-fourths of the calibration standard's back-calculated concentrations must be within ± 20% (± 25% for LLOQ) of their individual target concentrations. A calibration standard will be considered a statistical outlier if the back-calculated concentration is greater than two times the acceptance criteria for that standard.

Lower Limit of Quantitation

The back-calculated concentrations of at least one of the duplicate lowest points in the calibration curve must be within ± 25% of the target concentration for PFOSA and PFOSAA to qualify as the LLOQ and within ± 20% of the target concentration to qualify as the LLOQ for all other analytes. If this criterion is not met, the next level is subjected to the same test and the LLOQ raised accordingly.

Quality Control Samples

Each analytical run will include low, medium and high QC samples in duplicate. The measured concentrations of at least two-thirds of all analytical QCs must be within ± 20% of their target concentrations (± 25% for PFOSA and PFOSAA), and no two QCs at the same concentration can be outside the limit.

Based upon the results, the method is suitable for use with human plasma.

7. ADDITIONAL POST-REVALIDATION EXPERIMENTS

Additional experiments were conducted subsequent to completion of the revalidation to further elucidate the applicability of the revalidated method to sample analysis. These tests were performed outside of any revalidation or sample analysis studies.

7.1. Ion Suppression Experiment

During analysis of medical surveillance serum samples, it was noted that the internal standard response for the study samples differed from the standards and quality controls. POAA and the internal standard (THPFOS) have very similar retention

times. Therefore, THPFOS and POAA are not chromatographically resolved in extracted samples containing high concentrations of POAA. This co-elution can suppress the internal standard response and introduce a (high) bias in the measured concentration of analytes when compared against the standard curve. The suppression of the internal standard signal is proportional to the concentration of POAA in the sample and dependent on the LC resolution of the two compounds.

An experiment was performed to evaluate the magnitude of this effect. Low control samples were fortified with various (high) levels of POAA. The test samples were analyzed in triplicate in a single run (PFOSMD-41). The mean concentration of the test samples were then compared against the mean of the analytical low control samples. A summary of the results is shown in the following table:

	PFOS	*PFOSA*	*PFOSAA*	*PFHS*	*M556*	*M570*	*IS Area*
Control Mean Conc. (ppb)	51.8	4.05	6.83	8.04	5.41	6.87	108857
POAA added at ~59200 ppb							
% change from control mean	182%	152%	180%	180%	216%	207%	-75%
POAA added at ~11800 ppb							
% change from control mean	28%	39%	52%	29%	52%	56%	-43%
POAA added at ~5920 ppb							
% change from control mean	16%	21%	30%	16%	36%	34%	-32%
POAA added at ~592 ppb							
% change from control mean	-2%	-11%	-5%	-7%	-8%	-8%	+11%

The experiment verified that very high levels of POAA resulted in a lower response for the internal standard, which then results in a falsely elevated concentration for the other 6 analytes. No significant bias is introduced when the extracted concentration of POAA is within the revalidated range of the analytical method (approximately 500 ppb). However, some samples can contain POAA at concentrations above the revalidated range of the analytical method, and will likely introduce a bias in the reported concentrations of PFOS, PFHS, PFOSA, PFOSAA, M556, or M570 when the concentration of these analytes are determined prior to sample dilution. The amount of bias could range up to approximately 56%. However, the amount of

PFOS, PFHS, PFOSA, PFOSAA, M556, or M570 impurities in the POAA reference material is not known. This prevents accurate determination of the % bias. It should be noted that since the amount of ion suppression is dependent upon the amount of extracted POAA, no concentration bias is expected for an analyte result determined from a pre-diluted sample wherein the extracted POAA concentration is less than approximately 500 ppb. This bias will not significantly effect the interpretation of the analytical results.

7.2. Dilution Matrix Experiment

The amount of Chinese plasma supplied by 3M is limited. Therefore, it cannot be used on a routine basis for dilution. Human serum (control serum) is used to dilute test samples as needed to obtain measurable concentrations within the range of the calibration curve. The measured concentrations of fluorochemicals (FC) obtained for diluted samples must then be corrected for persistent levels of the analytes in the control serum. Experiments were performed to determine whether a substitute matrix that did not contain persistent levels of FC analytes could be used when performing dilutions.

Previous experiments had shown that samples diluted with buffer instead of serum did not accurately quantitate against a typical serum curve (data not reported). This indicated that the matrix to buffer ratio of the sample affected the extraction recovery. For the experiments described in this report, QC samples were diluted with buffer. The calibration curve was prepared in the same buffer to matrix ratio as the QC samples to determine if this was a viable method for diluting samples. A different dilution ratio was prepared to determine if samples could be quantitated from a curve prepared in a matrix to buffer ratio that was different from the QC samples (e.g., if QC samples diluted with matrix by a factor of 50 (2% matrix in buffer) could be quantitated against a curve prepared in 10% matrix in buffer). For the experiments described below, ammonium acetate buffer was used to dilute the matrix for the QCs and calibration curves.

Calibration Curve and QC Preparation

The experiment (PFOSMD run 39) used a curve prepared in 10% serum in buffer, and three levels of QC samples diluted with buffer by a factor of 10 (10% matrix to buffer ratio). The QC concentrations prior to dilution were 150, 400, and 4000 ppb plus the persistent level for each analyte. QC samples at 4000 ppb (plus persistent level) were also diluted 1:50 with buffer.

The calibration curve parameter summaries and individual calibration standard results for the runs can be found in Tables 99 – 112. The quality control sample results can be found in Tables 113 – 119.

Results for 10% Serum in Buffer (PFOSMD Run 39)

The mean accuracy (% theoretical) for all analytes at all QC concentration levels diluted with buffer by a factor of 10 (the same serum to buffer ratio as the curve) ranged from 94.1% to 114.3%. The intra-assay precision (%CV) for all analytes were less than or equal to 5.6% for each QC concentration.

The mean accuracy (% theoretical) for the QC samples diluted with buffer by a factor of 50 ranged from 56.5% to 84.8%. The inter-assay precision (%CV) for all analytes were less than or equal to 4.4%. All analytes were biased low, and all analytes except for POAA and PFHS were not within ±20% of the target concentrations.

QC samples met the acceptance criteria of ± 20% of target concentration (±25% for PFOSAA) when diluted with buffer if quantitated against a curve prepared in the same serum to buffer ratio. QC samples diluted with buffer at a different ratio than the calibration curve did not meet the acceptance criteria for all analytes.

It appears that quantitating samples diluted with buffer against a curve prepared in the same serum to buffer ratio is a viable way minimizing the corrections needed due to persistent levels of analytes in the dilution matrix. However, since most analytes cannot be accurately quantitated against a calibration curve that is prepared in a serum/buffer ratio different from that of the diluted samples, a separate curve with the same buffer to matrix ratio would need to be prepared for each dilution factor needed.

7.3. Serum and Plasma Comparison

Because the choice of calibration curve and quality control matrix is dependent upon the desired LLOQ, it is not always possible to match the subject sample matrix with that of the calibration curve and quality control matrix. For instance, it is necessary to use Chinese plasma to prepare the calibration standards and quality control samples when analyzing serum samples where a LLOQ of ≤ 5.00 ppb is desired. Therefore, to evaluate if, and to what extent, the use of plasma calibration standards affects the quantitative analysis of fluorochemicals in serum, a comparison of quantitative results obtained from plasma curves versus serum curves was performed.

Calibration curves were prepared in both human serum and Chinese plasma, and were analyzed along with serum and Chinese plasma quality control samples in replicates of four. The results for the calibration and quality control samples are shown in Tables 120 – 140.

The confidence interval around the serum QC mean was determined for serum samples quantitated against the plasma curve using Microsoft® Excel 97. Alpha (α) was set to 0.05 for a 95% confidence level. The confidence interval around the mean was compared to the analytical acceptance range of the QC which is ± 20% (± 25% for PFOSA and PFOSAA) of the target concentration. The summary results of the experiment are shown in Table 141.

The 10/27/00 serum curve for M570 had a raised LLOQ of 5.20 ppb. Data for PFOSA and PFOSAA analyzed on 10/27/00 and data for PFHS and M556 analyzed on 4/25/01 are not used because either the serum or plasma calibration curve or the QC samples did not meet the acceptance criteria. One high QC replicate analyzed on 10/27/00 was not included in the statistical calculations because of an extraction error.

The confidence intervals around the mean serum QC results for PFOS are within the acceptance criteria at all levels.

The confidence intervals around the mean serum QC results for PFOSA are greater than the acceptance range of ±25% deviation. The confidence intervals ranged up to 78% deviation from target.

The confidence intervals around the mean serum medium QC result for PFOSAA was 33.9% deviation which is greater than the plasma acceptance range of ±25% deviation. The confidence interval for the PFOSAA low and high QC samples are within the acceptance criteria.

The confidence intervals around the mean serum QC results for POAA and PFHS are greater than the acceptance range of ±20% deviation. The confidence intervals ranged up to 27.4% deviation from target for POAA and up to 37.6% deviation from target for PFHS.

The confidence interval around the mean serum low QC results for M556 was –26.9% deviation. This is greater than the acceptance range of ±20% deviation and may be due a low value obtained for one replicate. The confidence intervals for the M556 medium and high QC samples are within the acceptance criteria.

The confidence intervals around the mean serum medium QC result for M570 was 21.9% deviation which is slightly greater than the plasma acceptance range of ±20% deviation. The confidence interval for the M570 low and high QC samples are within the acceptance criteria.

CONCLUSION

Serum sample results obtained using plasma curves for PFOSA, PFOSAA, POAA, PFHS, M556, and M570 did not meet NWB SOP requirements for validation. Results may vary on average up to 26% from QC results obtained using human serum calibration curves at some concentration levels for PFHS, PFOSAA, POAA, M556, and M570. For PFOSA, results may vary on average up to 43% from QC results obtained using human serum calibration curves. However, when a lower limit of

quantitation less than 5 ppb is required by the Sponsor, it will be necessary to use plasma curves.

8. DATA MANAGEMENT

PFOS, PFOSA, PFOSAA, POAA, PFHS, M556, M570, and THPFOS chromatographic peaks were integrated using PE Sciex MacQuan software (version 1.6) with a smooth factor of one. Quantitation was based upon quadratic regression analysis of calibration curves (weighted $1/x^2$) using the area ratio vs. concentration calculated by the Watson® DMLIMS software (versions 6.1.1.03 and 6.1.1.04).

9. REFERENCES

[9.1.] C. Sakashita. "Quantitative Determination of PFOS, PFOSA, PFOSAA, POAA, PFHS, M556 and M570 in Human Serum by LC/MS/MS." Assay Revalidation Report. NWB Study NWBS00-040. NWB Report NWBR00-108. January 24, 2001.

[9.2.] D. Vollmer. "Quantitative Determination of PFOS, PFOSA, PFOSAA, N-MeFOSE-OH, N-EtFOSE-OH, POAA and PFHS in Human Serum by LC/MS/MS." Assay Validation Report. NWB Study NWBS98-082. NWB Report NWBR99-005. May 13, 1999.

10. DATA RETENTION

The raw data and final report for this study will be stored in the NWB Archive, 1121 East 3900 South, Salt Lake City, UT 84124 per regulations and contract agreement. 3M Environmental Technology and Services will be notified concerning final disposition of records at completion of contract obligations.

Table 1. Summary of Calibration Curve Parameters for PFOS in Human Serum

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
03-Jul-2000	23	-0.000004	0.019854	0.112640	0.9862	48.1	547
21-Aug-2000	18	0.000001	0.028989	0.257857	0.9849	48.1	547

A, B, and C are coefficients used to define the calibration curve.

Table 2. Summary of Calibration Curve Parameters for PFOSA in Human Serum

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
03-Jul-2000	23	-0.000017	0.054058	0.006149	0.9952	1.00	502
21-Aug-2000	18	0.000005	0.082782	0.019970	0.9952	1.00	502

A, B, and C are coefficients used to define the calibration curve.

Table 3. Summary of Calibration Curve Parameters for PFOSAA in Human Serum

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
03-Jul-2000	23	-0.000002	0.013656	-0.020557	0.9903	6.00	505
21-Aug-2000	18	0.000005	0.025511	-0.020269	0.9978	6.00	505

A, B, and C are coefficients used to define the calibration curve.

Table 4. Summary of Calibration Curve Parameters for POAA in Human Serum

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
03-Jul-2000	23	-0.000002	0.013683	0.010580	0.9979	5.76	505
21-Aug-2000	18	0.000003	0.013704	0.017950	0.9966	5.76	505

A, B, and C are coefficients used to define the calibration curve.

Table 5. Summary of Calibration Curve Parameters for PFHS in Human Serum

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
03-Jul-2000	23	-0.000006	0.018323	0.012882	0.9971	3.15	502
21-Aug-2000	18	-0.000001	0.022486	0.017181	0.9970	3.15	502

A, B, and C are coefficients used to define the calibration curve.

Table 6. Summary of Calibration Curve Parameters for M556 in Human Serum

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
03-Jul-2000	23	0.000000	0.002714	0.000585	0.9925	4.30	502
21-Aug-2000	18	0.000001	0.011156	0.005643	0.9958	4.30	502

A, B, and C are coefficients used to define the calibration curve.

Table 7. Summary of Calibration Curve Parameters for M570 in Human Serum

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
03-Jul-2000	23	0.000000	0.003818	-0.004160	0.9963	5.60	505
21-Aug-2000	18	0.000005	0.036404	-0.017720	0.9977	5.60	505

A, B, and C are coefficients used to define the calibration curve.

Table 8. Back-Calculated Concentrations of Calibration Standards for PFOS in Human Serum

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	48.1	49.6	57.1	72.1	97.1	147	297	447	547
03-Jul-2000	23	50.7	52.1	64.0	77.3	98.4	158	309	472	564
		45.8	45.6	53.4	68.2	86.6	138	298	435	508
21-Aug-2000	18	51.7	54.3	60.3	80.5	101	152	304	489	566
		44.1	45.6	50.5	70.5	91.1	139	286	417	521

Table 9. Back-Calculated Concentrations of Calibration Standards for PFOSA in Human Serum

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	1.00	2.51	10.1	25.1	50.3	100	251	402	502
03-Jul-2000	23	1.10	2.54	10.6	27.3	51.6	103	259	419	534
		0.918	2.38	9.36	24.6	46.2	95.4	265	398	436
21-Aug-2000	18	1.01	2.73	10.4	27.9	52.7	106	253	416	530
		0.990	2.30	9.62	25.1	44.7	95.8	229	378	502

Table 10. Back-Calculated Concentrations of Calibration Standards for PFOSAA in Human Serum

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	6.00	7.50	15.0	30.0	55.0	105	255	405	505
03-Jul-2000	23	6.74	7.73	16.6	31.3	57.6	111	278	446	524
		5.49	6.93	13.6	27.3	52.0	99.6	249	382	451
21-Aug-2000	18	5.83	7.71	14.5	29.8	53.7	99.3	239	397	499
		6.13	7.50	14.4	32.2	57.0	108	268	414	513

Table 11. Back-Calculated Concentrations of Calibration Standards for POAA in Human Serum

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	5.76	7.26	14.8	29.8	54.8	105	255	405	505
03-Jul-2000	23	5.93	7.11	15.2	30.6	52.0	109	265	406	524
		5.74	6.93	15.6	29.4	53.1	99.6	261	404	474
21-Aug-2000	18	5.98	7.19	13.9	28.2	52.8	102	256	401	481
		6.06	6.58	15.2	32.5	55.2	108	270	412	511

Table 12. Back-Calculated Concentrations of Calibration Standards for PFHS in Human Serum

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	3.15	4.65	12.2	27.2	52.2	102	252	402	502
03-Jul-2000	23	3.13	4.45	12.8	27.7	49.3	103	256	405	515
		3.20	4.55	13.7	26.3	49.1	99.7	253	402	487
21-Aug-2000	18	2.85	4.72	11.8	28.1	48.8	98.3	245	408	497
		3.17	5.23	12.1	27.9	51.9	105	258	404	502

Table 13. Back-Calculated Concentrations of Calibration Standards for M556 in Human Serum

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	4.30	11.8	26.8	51.8	102	252	402	502
03-Jul-2000	23	4.44	12.6	28.9	53.2	113	274	434	528
		4.18	11.1	23.8	47.5	95.1	247	381	448
21-Aug-2000	18	4.36	11.0	25.5	49.0	101	234	391	469
		4.35	11.8	27.2	55.4	106	284	415	516

Table 14. Back-Calculated Concentrations of Calibration Standards for M570 in Human Serum

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	5.60	7.10	14.6	29.6	54.6	105	255	405	505
03-Jul-2000	23	6.16	6.90	15.4	29.8	54.0	107	273	414	510
		5.54	6.43	14.3	28.7	52.6	104	265	404	464
21-Aug-2000	18	5.11	7.35	13.7	30.2	53.3	101	249	401	503
		5.84	7.43	14.4	31.1	54.5	106	269	406	505

Table 15. Quality Control Samples for PFOS in Human Serum

Mean of replicate QCs reported. All concentrations are expressed as ppb.

Run Date	Run Number	Low QC 51.1 ppb	*%Dif.*	Medium QC 197 ppb	*%Dif.*	High QC 446 ppb	*%Dif.*
03-Jul-2000	23	47.7	*-6.65*	168	*-14.7*	382	*-14.3*
21-Aug-2000	18	46.7	*-8.61*	170	*-13.7*	378	*-15.2*

Table 16. Quality Control Samples for PFOSA in Human Serum

Mean of replicate QCs reported. All concentrations are expressed as ppb.

Run Date	Run Number	Low QC 4.00 ppb	*%Dif.*	Medium QC 150 ppb	*%Dif.*	High QC 400 ppb	*%Dif.*
03-Jul-2000	23	2.73	*-31.8*	100	*-33.3*	286	*-28.5*
21-Aug-2000	18	4.34	*8.50*	150	*0.00*	395	*-1.25*

Table 17. Quality Control Samples for PFOSAA in Human Serum

Mean of replicate QCs reported. All concentrations are expressed as ppb.

Run Date	Run Number	Low QC 9.00 ppb	*%Dif.*	Medium QC 155 ppb	*%Dif.*	High QC 405 ppb	*%Dif.*
03-Jul-2000	23	7.73	*-14.1*	110	*-29.0*	288	*-28.9*
21-Aug-2000	18	9.95	*10.6*	160	*3.23*	413	*1.98*

Table 18. Quality Control Samples for POAA in Human Serum

Mean of replicate QCs reported. All concentrations are expressed as ppb.

Run Date	Run Number	Low QC 8.74 ppb	*%Dif.*	Medium QC 154 ppb	*%Dif.*	High QC 403 ppb	*%Dif.*
03-Jul-2000	23	8.45	*-3.32*	150	*-2.60*	400	*-0.744*
21-Aug-2000	18	7.82	*-10.5*	149	*-3.25*	372	*-7.69*

Table 19. Quality Control Samples for PFHS in Human Serum

Mean of replicate QCs reported. All concentrations are expressed as ppb.

Run Date	Run Number	Low QC 6.15 ppb	*%Dif.*	Medium QC 152 ppb	*%Dif.*	High QC 402 ppb	*%Dif.*
03-Jul-2000	23	6.24	*1.46*	157	*3.29*	427	*6.22*
21-Aug-2000	18	6.86	*11.5*	164	*7.89*	403	*0.249*

Table 20. Quality Control Samples for M556 in Human Serum

Mean of replicate QCs reported. All concentrations are expressed as ppb.

Run Date	Run Number	Low QC 5.80 ppb	*%Dif.*	Medium QC 152 ppb	*%Dif.*	High QC 402 ppb	*%Dif.*
03-Jul-2000	23	5.52	*-4.83*	145	*-4.61*	381	*-5.22*
21-Aug-2000	18	5.42	*-6.55*	141	*-7.24*	367	*-8.71*

Table 21. Quality Control Samples for M570 in Human Serum

Mean of replicate QCs reported. All concentrations are expressed as ppb.

Run Date	Run Number	Low QC 8.60 ppb	*%Dif.*	Medium QC 155 ppb	*%Dif.*	High QC 405 ppb	*%Dif.*
03-Jul-2000	23	8.20	*-4.65*	146	*-5.81*	390	*-3.70*
21-Aug-2000	18	8.02	*-6.74*	149	*-3.87*	386	*-4.69*

Table 22. Freeze-Thaw and Room Temperature Matrix Stability for PFOS in Human Serum

All concentrations are expressed as ppb.

Run Number	18
Run Date	21-Aug-2000
	LOW QC
	After 7 Freeze-Thaw cycles
	46.8
	45.1
	44.0
Mean	45.3
S.D.	1.41
% CV	3.1
Control (ng/mL)	46.7
Mean % Dev. from Control	-3.0
	MEDIUM QC
	After 7 Freeze-Thaw cycles
	170
	158
	164
Mean	164
S.D.	6.00
% CV	3.7
Control (ng/mL)	170
Mean % Dev. from Control	-3.5
	HIGH QC
	After 7 Freeze-Thaw cycles
	369
	376
	361
Mean	369
S.D.	7.51
% CV	2.0
Control (ng/mL)	378
Mean % Dev. from Control	-2.4

Control = mean of analytical QC samples analyzed in the same run
NOTE: Samples were left at room temperature for up to 6.75 hour periods between each freeze cycle

Table 23. Freeze-Thaw and Room Temperature Matrix Stability for PFOSA in Human Serum

All concentrations are expressed as ppb.

Run Number	**18**
Run Date	**21-Aug-2000**
	LOW QC
	After 7 Freeze-Thaw cycles
	3.73
	3.53
	3.48
Mean	3.58
S.D.	0.132
% CV	3.7
Control (ng/mL)	4.34
Mean % Dev. from Control	-17.5
	MEDIUM QC
	After 7 Freeze-Thaw cycles
	108
	126
	125
Mean	120
S.D.	10.1
% CV	8.4
Control (ng/mL)	150
Mean % Dev. from Control	-20.0
	HIGH QC
	After 7 Freeze-Thaw cycles
	348
	361
	350
Mean	353
S.D.	7.00
% CV	2.0
Control (ng/mL)	395
Mean % Dev. from Control	-10.6

Control = mean of analytical QC samples analyzed in the same run
NOTE: Samples were left at room temperature for up to 6.75 hour periods between each freeze cycle

Table 24. Freeze-Thaw Stability and Room Temperature Matrix for PFOSAA in Human Serum

All concentrations are expressed as ppb.

Run Number	**18**
Run Date	**21-Aug-2000**
	LOW QC
	After 7 Freeze-Thaw cycles
	9.08
	9.85
	9.46
Mean	9.46
S.D.	0.385
% CV	4.1
Control (ng/mL)	9.95
Mean % Dev. from Control	-4.9
	MEDIUM QC
	After 7 Freeze-Thaw cycles
	114
	147
	147
Mean	136
S.D.	19.1
% CV	14.0
Control (ng/mL)	160
Mean % Dev. from Control	-15.0
	HIGH QC
	After 7 Freeze-Thaw cycles
	383
	403
	391
Mean	392
S.D.	10.1
% CV	2.6
Control (ng/mL)	413
Mean % Dev. from Control	-5.1

Control = mean of analytical QC samples analyzed in the same run
NOTE: Samples were left at room temperature for up to 6.75 hour periods between each freeze cycle

Table 25. Freeze-Thaw and Room Temperature Matrix Stability for POAA in Human Serum

All concentrations are expressed as ppb.

Run Number	18
Run Date	21-Aug-2000
	LOW QC
	After 7 Freeze-Thaw cycles
	8.06
	8.83
	8.25
Mean	8.38
S.D.	0.401
% CV	4.8
Control (ng/mL)	7.82
Mean % Dev. from Control	7.2
	MEDIUM QC
	After 7 Freeze-Thaw cycles
	151
	149
	154
Mean	151
S.D.	2.52
% CV	1.7
Control (ng/mL)	149
Mean % Dev. from Control	1.3
	HIGH QC
	After 7 Freeze-Thaw cycles
	364
	379
	372
Mean	372
S.D.	7.51
% CV	2.0
Control (ng/mL)	372
Mean % Dev. from Control	0.0

Control = mean of analytical QC samples analyzed in the same run
NOTE: Samples were left at room temperature for up to 6.75 hour periods between each freeze cycle

Table 26. Freeze-Thaw and Room Temperature Matrix Stability for PFHS in Human Serum

All concentrations are expressed as ppb.

Run Number	**18**
Run Date	**21-Aug-2000**
	LOW QC
	After 7 Freeze-Thaw cycles
	6.05
	6.27
	7.12
Mean	6.48
S.D.	0.565
% CV	8.7
Control (ng/mL)	6.86
Mean % Dev. from Control	-5.5
	MEDIUM QC
	After 7 Freeze-Thaw cycles
	165
	157
	163
Mean	162
S.D.	4.16
% CV	2.6
Control (ng/mL)	164
Mean % Dev. from Control	-1.2
	HIGH QC
	After 7 Freeze-Thaw cycles
	397
	417
	405
Mean	406
S.D.	10.1
% CV	2.5
Control (ng/mL)	403
Mean % Dev. from Control	0.7

Control = mean of analytical QC samples analyzed in the same run
NOTE: Samples were left at room temperature for up to 6.75 hour periods between each freeze cycle

Table 27. Freeze-Thaw and Room Temperature Matrix Stability for M556 in Human Serum

All concentrations are expressed as ppb.

Run Number	**18**
Run Date	**21-Aug-2000**
	LOW QC
	After 7 Freeze-Thaw cycles
	4.95
	5.07
	5.09
Mean	5.04
S.D.	0.0757
% CV	1.5
Control (ng/mL)	5.42
Mean % Dev. from Control	-7.0
	MEDIUM QC
	After 7 Freeze-Thaw cycles
	128
	140
	135
Mean	134
S.D.	6.03
% CV	4.5
Control (ng/mL)	141
Mean % Dev. from Control	-5.0
	HIGH QC
	After 7 Freeze-Thaw cycles
	359
	372
	374
Mean	368
S.D.	8.14
% CV	2.2
Control (ng/mL)	367
Mean % Dev. from Control	0.3

Control = mean of analytical QC samples analyzed in the same run
NOTE: Samples were left at room temperature for up to 6.75 hour periods between each freeze cycle

Table 28. Freeze-Thaw and Room Temperature Matrix Stability for M570 in Human Serum

All concentrations are expressed as ppb.

Run Number	**18**
Run Date	**21-Aug-2000**
	LOW QC
	After 7 Freeze-Thaw cycles
	7.68
	8.76
	8.51
Mean	8.32
S.D.	0.565
% CV	6.8
Control (ng/mL)	8.02
Mean % Dev. from Control	**3.7**
	MEDIUM QC
	After 7 Freeze-Thaw cycles
	142
	142
	143
Mean	142
S.D.	0.577
% CV	0.4
Control (ng/mL)	149
Mean % Dev. from Control	**-4.7**
	HIGH QC
	After 7 Freeze-Thaw cycles
	356
	371
	370
Mean	366
S.D.	8.39
% CV	2.3
Control (ng/mL)	386
Mean % Dev. from Control	**-5.2**

Control = mean of analytical QC samples analyzed in the same run
NOTE: Samples were left at room temperature for up to 6.75 hour periods between each freeze cycle

Table 29. Reinjection Stability for PFOS

All concentrations are expressed as ppb.

Run Number	**23**
Run Date	**3-Jul-2000**
	LOW QC
	Reinjection
	(After 2.5 Days Room Temp.)
	49.1
	48.0
	50.0
	46.1
	45.1
Mean	47.7
S.D.	2.04
% CV	4.3
Control (ng/mL)	47.2
Mean % Dev. from Control	**1.1**
	MEDIUM QC
	Reinjection
	(After 2.5 Days Room Temp.)
	164
	172
	170
	164
	170
Mean	168
S.D.	3.74
% CV	2.2
Control (ng/mL)	173
Mean % Dev. from Control	**-2.9**
	HIGH QC
	Reinjection
	(After 2.5 Days Room Temp.)
	391
	369
	368
	372
	411
Mean	382
S.D.	18.6
% CV	4.9
Control (ng/mL)	393
Mean % Dev. from Control	**-2.8**

Control = mean of analytical QC samples analyzed in run 15 (original injection)

Table 30. Reinjection Stability for PFOSA

All concentrations are expressed as ppb.

Run Number	**23**
Run Date	**3-Jul-2000**
	LOW QC
	Reinjection
	(After 2.5 Days Room Temp.)
	2.79
	2.69
	2.85
	2.66
	2.68
Mean	2.73
S.D.	0.0820
% CV	3.0
Control (ng/mL)	2.82
Mean % Dev. from Control	-3.2
	MEDIUM QC
	Reinjection
	(After 2.5 Days Room Temp.)
	102
	102
	97.9
	94.4
	105
Mean	100
S.D.	4.14
% CV	4.1
Control (ng/mL)	103
Mean % Dev. from Control	-2.9
	HIGH QC
	Reinjection
	(After 2.5 Days Room Temp.)
	275
	273
	290
	284
	307
Mean	286
S.D.	13.7
% CV	4.8
Control (ng/mL)	300
Mean % Dev. from Control	-4.7

Control = mean of analytical QC samples analyzed in run 15 (original injection)

Table 31. Reinjection Stability for PFOSAA

All concentrations are expressed as ppb.

Run Number	**23**
Run Date	**3-Jul-2000**
	LOW QC
	Reinjection
	(After 2.5 Days Room Temp.)
	7.78
	7.58
	8.15
	7.41
	7.74
Mean	7.73
S.D.	0.276
% CV	3.6
Control (ng/mL)	7.59
Mean % Dev. from Control	**1.8**
	MEDIUM QC
	Reinjection
	(After 2.5 Days Room Temp.)
	109
	112
	113
	104
	110
Mean	110
S.D.	3.51
% CV	3.2
Control (ng/mL)	113
Mean % Dev. from Control	**-2.7**
	HIGH QC
	Reinjection
	(After 2.5 Days Room Temp.)
	287
	285
	284
	278
	305
Mean	288
S.D.	10.2
% CV	3.5
Control (ng/mL)	301
Mean % Dev. from Control	**-4.3**

Control = mean of analytical QC samples analyzed in run 15 (original injection)

Table 32. Reinjection Stability for POAA

All concentrations are expressed as ppb.

Run Number	**23**
Run Date	**3-Jul-2000**
	LOW QC
	Reinjection
	(After 2.5 Days Room Temp.)
	8.68
	8.43
	8.31
	8.41
	8.41
Mean	8.45
S.D.	0.138
% CV	1.6
Control (ng/mL)	8.10
Mean % Dev. from Control	4.3
	MEDIUM QC
	Reinjection
	(After 2.5 Days Room Temp.)
	150
	152
	156
	144
	147
Mean	150
S.D.	4.60
% CV	3.1
Control (ng/mL)	144
Mean % Dev. from Control	4.2
	HIGH QC
	Reinjection
	(After 2.5 Days Room Temp.)
	417
	408
	386
	388
	401
Mean	400
S.D.	13.2
% CV	3.3
Control (ng/mL)	384
Mean % Dev. from Control	4.2

Control = mean of analytical QC samples analyzed in run 15 (original injection)

Table 33. Reinjection Stability for PFHS

All concentrations are expressed as ppb.

Run Number	**23**
Run Date	**3-Jul-2000**
	LOW QC
	Reinjection
	(After 2.5 Days Room Temp.)
	6.30
	6.32
	6.48
	5.98
	6.11
Mean	6.24
S.D.	0.195
% CV	3.1
Control (ng/mL)	6.22
Mean % Dev. from Control	**0.3**
	MEDIUM QC
	Reinjection
	(After 2.5 Days Room Temp.)
	153
	156
	156
	159
	159
Mean	157
S.D.	2.51
% CV	1.6
Control (ng/mL)	158
Mean % Dev. from Control	**-0.6**
	HIGH QC
	Reinjection
	(After 2.5 Days Room Temp.)
	441
	416
	421
	409
	450
Mean	427
S.D.	17.4
% CV	4.1
Control (ng/mL)	445
Mean % Dev. from Control	**-4.0**

Control = mean of analytical QC samples analyzed in run 15 (original injection)

Table 34. Reinjection Stability for M556

All concentrations are expressed as ppb.

Run Number	**23**
Run Date	**3-Jul-2000**
	LOW QC
	Reinjection
	(After 2.5 Days Room Temp.)
	5.78
	5.73
	5.45
	5.29
	5.33
Mean	5.52
S.D.	0.227
% CV	4.1
Control (ng/mL)	5.61
Mean % Dev. from Control	-1.6
	MEDIUM QC
	Reinjection
	(After 2.5 Days Room Temp.)
	144
	147
	147
	143
	146
Mean	145
S.D.	1.82
% CV	1.3
Control (ng/mL)	147
Mean % Dev. from Control	-1.4
	HIGH QC
	Reinjection
	(After 2.5 Days Room Temp.)
	388
	373
	381
	367
	398
Mean	381
S.D.	12.2
% CV	3.2
Control (ng/mL)	396
Mean % Dev. from Control	-3.8

Control = mean of analytical QC samples analyzed in run 15 (original injection)

Table 35. Reinjection Stability for M570

All concentrations are expressed as ppb.

Run Number	**23**
Run Date	**3-Jul-2000**
	LOW QC
	Reinjection
	(After 2.5 Days Room Temp.)
	8.31
	8.36
	8.52
	8.10
	7.71
Mean	8.20
S.D.	0.312
% CV	3.8
Control (ng/mL)	8.31
Mean % Dev. from Control	-1.3
	MEDIUM QC
	Reinjection
	(After 2.5 Days Room Temp.)
	145
	149
	146
	144
	145
Mean	146
S.D.	1.92
% CV	1.3
Control (ng/mL)	147
Mean % Dev. from Control	-0.7
	HIGH QC
	Reinjection
	(After 2.5 Days Room Temp.)
	391
	384
	390
	380
	405
Mean	390
S.D.	9.51
% CV	2.4
Control (ng/mL)	401
Mean % Dev. from Control	-2.7

Control = mean of analytical QC samples analyzed in run 15 (original injection)

Table 36. Room Temperature Extract Stability for PFOS

All concentrations are expressed as ppb.

Run Number	**18**
Run Date	**21-Aug-2000**
	LOW QC *After 7 Days*
	51.7
	46.0
	46.2
Mean	48.0
S.D.	3.23
% CV	6.7
Control (ng/mL)	46.7
Mean % Dev. from Control	**2.8**
	HIGH QC *After 7 Days*
	373
	402
	397
Mean	391
S.D.	15.5
% CV	4.0
Control (ng/mL)	378
Mean % Dev. from Control	**3.4**

Control = mean of QC samples analyzed in the same run immediately following extraction

Table 37. Room Temperature Extract Stability for PFOSA

All concentrations are expressed as ppb.

Run Number	**18**
Run Date	**21-Aug-2000**
	LOW QC *After 7 Days*
	3.48
	3.34
	3.30
Mean	3.37
S.D.	0.0945
% CV	2.8
Control (ng/mL)	3.13
Mean % Dev. from Control	7.7
	HIGH QC *After 7 Days*
	337
	362
	334
Mean	344
S.D.	15.4
% CV	4.5
Control (ng/mL)	322
Mean % Dev. from Control	**6.8**

Control = mean of QC samples analyzed in the same run immediately following extraction

Table 38. Room Temperature Extract Stability for PFOSAA

All concentrations are expressed as ppb.

Run Number	**18**
Run Date	**21-Aug-2000**
	LOW QC *After 7 Days*
	8.2
	9.2
	9.3
Mean	8.89
S.D.	0.607
% CV	6.8
Control (ng/mL)	8.02
Mean % Dev. from Control	**10.8**
	HIGH QC *After 7 Days*
	321
	337
	343
Mean	334
S.D.	11.4
% CV	3.4
Control (ng/mL)	297
Mean % Dev. from Control	**12.5**

Control = mean of QC samples analyzed in the same run immediately following extraction

Table 39. Room Temperature Extract Stability for POAA

All concentrations are expressed as ppb.

Run Number	**18**
Run Date	**21-Aug-2000**
	LOW QC *After 7 Days*
	6.92
	7.98
	8.15
Mean	7.68
S.D.	0.667
% CV	8.7
Control (ng/mL)	7.82
Mean % Dev. from Control	-1.8
	HIGH QC *After 7 Days*
	360
	395
	378
Mean	378
S.D.	17.5
% CV	4.6
Control (ng/mL)	372
Mean % Dev. from Control	1.6

Control = mean of QC samples analyzed in the same run immediately following extraction

Table 40. Room Temperature Extract Stability for PFHS

All concentrations are expressed as ppb.

Run Number	**18**
Run Date	**21-Aug-2000**
	LOW QC *After 7 Days*
	6.31
	6.21
	5.90
Mean	6.14
S.D.	0.214
% CV	3.5
Control (ng/mL)	6.86
Mean % Dev. from Control	**-10.5**
	HIGH QC *After 7 Days*
	396
	405
	412
Mean	404
S.D.	8.02
% CV	2.0
Control (ng/mL)	403
Mean % Dev. from Control	**0.2**

Control = mean of QC samples analyzed in the same run immediately following extraction

Table 41. Room Temperature Extract Stability for M556

All concentrations are expressed as ppb.

Run Number	**18**
Run Date	**21-Aug-2000**
	LOW QC *After 7 Days*
	5.13
	5.65
	5.50
Mean	5.43
S.D.	0.268
% CV	4.9
Control (ng/mL)	5.42
Mean % Dev. from Control	**0.2**
	HIGH QC *After 7 Days*
	373
	376
	387
Mean	379
S.D.	7.37
% CV	1.9
Control (ng/mL)	367
Mean % Dev. from Control	**3.3**

Control = mean of QC samples analyzed in the same run immediately following extraction

Table 42. Room Temperature Extract Stability for M570

All concentrations are expressed as ppb.

Run Number	**18**
Run Date	**21-Aug-2000**
	LOW QC *After 7 Days*
	8.86
	9.13
	8.74
Mean	8.91
S.D.	0.200
% CV	2.2
Control (ng/mL)	8.02
Mean % Dev. from Control	**11.1**
	HIGH QC *After 7 Days*
	409
	429
	423
Mean	420
S.D.	10.3
% CV	2.5
Control (ng/mL)	386
Mean % Dev. from Control	**8.8**

Control = mean of QC samples analyzed in the same run immediately following extraction

Table 43. Reduced Temperature (1 to 8 °C) Extract Stability for PFOS

All concentrations are expressed as ppb.

Run Number	**18**
Run Date	**21-Aug-2000**
	LOW QC
	1 to 8°C
	After 4 Days
	49.3
	45.6
	44.8
Mean	46.6
S.D.	2.40
% CV	5.2
Control (ng/mL)	46.7
Mean % Dev. from Control	**-0.2**
	HIGH QC
	1 to 8°C
	After 4 Days
	391
	385
	377
Mean	384
S.D.	7.02
% CV	1.8
Control (ng/mL)	378
Mean % Dev. from Control	**1.6**

Control = mean of QC samples analyzed in the same run immediately following extraction

Table 44. Reduced Temperature (1 to 8 °C) Extract Stability for PFOSA

All concentrations are expressed as ppb.

Run Number	**18**
Run Date	**21-Aug-2000**
	LOW QC
	1 to 8°C
	After 4 Days
	3.22
	2.79
	2.86
Mean	2.96
S.D.	0.231
% CV	7.8
Control (ng/mL)	3.13
Mean % Dev. from Control	**-5.4**
	HIGH QC
	1 to 8°C
	After 4 Days
	350
	297
	304
Mean	317
S.D.	28.8
% CV	9.1
Control (ng/mL)	322
Mean % Dev. from Control	**-1.6**

Control = mean of QC samples analyzed in the same run immediately following extraction

Table 45. Reduced Temperature (1 to 8 °C) Extract Stability for PFOSAA

All concentrations are expressed as ppb.

Run Number	**18**
Run Date	**21-Aug-2000**
	LOW QC
	1 to 8°C
	After 4 Days
	7.78
	8.55
	8.32
Mean	8.22
S.D.	0.395
% CV	4.8
Control (ng/mL)	8.02
Mean % Dev. from Control	**2.5**
	HIGH QC
	1 to 8°C
	After 4 Days
	312
	306
	311
Mean	310
S.D.	3.21
% CV	1.0
Control (ng/mL)	297
Mean % Dev. from Control	**4.4**

Control = mean of QC samples analyzed in the same run immediately following extraction

Table 46. Reduced Temperature (1 to 8 °C) Extract Stability for POAA

All concentrations are expressed as ppb.

Run Number	**18**
Run Date	**21-Aug-2000**
	LOW QC
	1 to 8°C
	After 4 Days
	7.78
	8.75
	8.39
Mean	8.31
S.D.	0.490
% CV	5.9
Control (ng/mL)	7.82
Mean % Dev. from Control	**6.3**
	HIGH QC
	1 to 8°C
	After 4 Days
	381
	394
	385
Mean	387
S.D.	6.66
% CV	1.7
Control (ng/mL)	372
Mean % Dev. from Control	**4.0**

Control = mean of QC samples analyzed in the same run immediately following extraction

Table 47. Reduced Temperature (1 to 8 °C) Extract Stability for PFHS

All concentrations are expressed as ppb.

Run Number	**18**
Run Date	**21-Aug-2000**
	LOW QC
	1 to 8°C
	After 4 Days
	6.76
	6.34
	7.74
Mean	6.95
S.D.	0.718
% CV	10.3
Control (ng/mL)	6.86
Mean % Dev. from Control	**1.3**
	HIGH QC
	1 to 8°C
	After 4 Days
	400
	411
	423
Mean	411
S.D.	11.5
% CV	2.8
Control (ng/mL)	403
Mean % Dev. from Control	**2.0**

Control = mean of QC samples analyzed in the same run immediately following extraction

Table 48. Reduced Temperature (1 to 8 °C) Extract Stability for M556

All concentrations are expressed as ppb.

Run Number	**18**
Run Date	**21-Aug-2000**
	LOW QC
	1 to 8°C
	After 4 Days
	5.47
	6.57
	6.82
Mean	6.29
S.D.	0.718
% CV	11.4
Control (ng/mL)	5.42
Mean % Dev. from Control	**16.1**
	HIGH QC
	1 to 8°C
	After 4 Days
	398
	404
	403
Mean	402
S.D.	3.21
% CV	0.8
Control (ng/mL)	367
Mean % Dev. from Control	**9.5**

Control = mean of QC samples analyzed in the same run immediately following extraction

Table 49. Reduced Temperature (1 to 8 °C) Extract Stability for M570

All concentrations are expressed as ppb.

Run Number	**18**
Run Date	**21-Aug-2000**
	LOW QC
	1 to 8°C
	After 4 Days
	7.96
	7.90
	9.21
Mean	8.36
S.D.	0.740
% CV	8.9
Control (ng/mL)	8.02
Mean % Dev. from Control	**4.2**
	HIGH QC
	1 to 8°C
	After 4 Days
	396
	394
	402
Mean	397
S.D.	4.16
% CV	1.0
Control (ng/mL)	386
Mean % Dev. from Control	**2.8**

Control = mean of QC samples analyzed in the same run immediately following extraction

Table 50. Reduced Temperature (-20 °C) Extract Stability for PFOS

All concentrations are expressed as ppb.

Run Number	**18**
Run Date	**21-Aug-2000**
	LOW QC -20°C *After 5 Days*
	47.2
	45.5
	40.9
Mean	44.5
S.D.	3.26
% CV	7.3
Control (ng/mL)	46.7
Mean % Dev. from Control	-4.7
	HIGH QC -20°C *After 5 Days*
	376
	373
	365
Mean	371
S.D.	5.69
% CV	1.5
Control (ng/mL)	378
Mean % Dev. from Control	-1.9

Control = mean of QC samples analyzed in the same run immediately following extraction

Table 51. Reduced Temperature (-20 °C) Extract Stability for PFOSA

All concentrations are expressed as ppb.

Run Number	**18**
Run Date	**21-Aug-2000**
	LOW QC
	-20°C
	After 5 Days
	2.95
	3.16
	3.14
Mean	3.08
S.D.	0.116
% CV	3.8
Control (ng/mL)	3.13
Mean % Dev. from Control	**-1.6**
	HIGH QC
	-20°C
	After 5 Days
	312
	312
	296
Mean	307
S.D.	9.24
% CV	3.0
Control (ng/mL)	322
Mean % Dev. from Control	**-4.7**

Control = mean of QC samples analyzed in the same run immediately following extraction

Table 52. Reduced Temperature (-20 °C) Extract Stability for PFOSAA

All concentrations are expressed as ppb.

Run Number	**18**
Run Date	**21-Aug-2000**
	LOW QC
	-20°C
	After 5 Days
	7.52
	8.32
	7.89
Mean	7.91
S.D.	0.400
% CV	5.1
Control (ng/mL)	8.02
Mean % Dev. from Control	-1.4
	HIGH QC
	-20°C
	After 5 Days
	294
	306
	287
Mean	296
S.D.	9.61
% CV	3.2
Control (ng/mL)	297
Mean % Dev. from Control	-0.3

Control = mean of QC samples analyzed in the same run immediately following extraction

Table 53. Reduced Temperature (-20 °C) Extract Stability for POAA

All concentrations are expressed as ppb.

Run Number	**18**
Run Date	**21-Aug-2000**
	LOW QC
	-20°C
	After 5 Days
	7.84
	10.2
	6.85
Mean	8.30
S.D.	1.72
% CV	20.7
Control (ng/mL)	7.82
Mean % Dev. from Control	**6.1**
	HIGH QC
	-20°C
	After 5 Days
	351
	373
	386
Mean	370
S.D.	17.7
% CV	4.8
Control (ng/mL)	372
Mean % Dev. from Control	**-0.5**

Control = mean of QC samples analyzed in the same run immediately following extraction

Table 54. Reduced Temperature (-20 °C) Extract Stability for PFHS

All concentrations are expressed as ppb.

Run Number	**18**
Run Date	**21-Aug-2000**
	LOW QC -20°C *After 5 Days*
	6.68
	6.06
	7.70
Mean	6.81
S.D.	0.828
% CV	12.2
Control (ng/mL)	6.86
Mean % Dev. from Control	-0.7
	HIGH QC -20°C *After 5 Days*
	403
	407
	417
Mean	409
S.D.	7.21
% CV	1.8
Control (ng/mL)	403
Mean % Dev. from Control	1.5

Control = mean of QC samples analyzed in the same run immediately following extraction

Table 55. Reduced Temperature (-20 °C) Extract Stability for M556

All concentrations are expressed as ppb.

Run Number	**18**
Run Date	**21-Aug-2000**
	LOW QC -20°C *After 5 Days*
	4.51
	5.69
	5.97
Mean	5.39
S.D.	0.775
% CV	14.4
Control (ng/mL)	5.42
Mean % Dev. from Control	**-0.6**
	HIGH QC -20°C *After 5 Days*
	358
	373
	378
Mean	370
S.D.	10.4
% CV	2.8
Control (ng/mL)	367
Mean % Dev. from Control	**0.8**

Control = mean of QC samples analyzed in the same run immediately following extraction

Table 56. Reduced Temperature (-20 °C) Extract Stability for M570

All concentrations are expressed as ppb.

Run Number	**18**
Run Date	**21-Aug-2000**
	LOW QC -20°C
	After 5 Days
	8.43
	8.52
	7.94
Mean	8.30
S.D.	0.312
% CV	3.8
Control (ng/mL)	8.02
Mean % Dev. from Control	3.5
	HIGH QC -20°C
	After 5 Days
	366
	388
	369
Mean	374
S.D.	11.9
% CV	3.2
Control (ng/mL)	386
Mean % Dev. from Control	-3.1

Control = mean of QC samples analyzed in the same run immediately following extraction

Table 57. Long-Term Matrix Stability for PFOS in Human Serum

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC 51.1 ppb 55 Days	Medium QC 197 ppb 55 Days	High QC 446 ppb 55 Days
21-Aug-2000	18	52.2	175	381
		50.1	171	391
		42.3	174	361
		43.8	166	371
		45.0	165	388
Mean		46.7	170	378
SD		4.26	4.55	12.4
%CV		9.1	2.7	3.3
%Bias		-8.6	-13.7	-15.2
N		5	5	5

Table 58. Long-Term Matrix Stability for PFOSA in Human Serum

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC 4.00 ppb 42 Days	Medium QC 150 ppb 42 Days	High QC 400 ppb 42 Days
21-Aug-2000	18	4.28	155	376
		4.56	158	420
		4.40	154	381
		4.12	142	397
		4.32	143	400
Mean		4.34	150	395
SD		0.161	7.37	17.4
%CV		3.7	4.9	4.4
%Bias		8.5	0.0	-1.3
N		5	5	5

Table 59. Long-Term Matrix Stability for PFOSAA in Human Serum

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC 9.00 ppb 42 Days	Medium QC 155 ppb 42 Days	High QC 405 ppb 42 Days
21-Aug-2000	18	9.64	157	403
		9.11	158	429
		10.5	164	406
		10.2	159	413
		10.3	160	416
Mean		9.95	160	413
SD		0.568	2.70	10.2
%CV		5.7	1.7	2.5
%Bias		10.6	3.2	2.0
N		5	5	5

Table 60. Long-Term Matrix Stability for POAA in Human Serum

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC 8.74 ppb 55 Days	Medium QC 154 ppb 55 Days	High QC 403 ppb 55 Days
21-Aug-2000	18	7.74	145	364
		7.54	138	380
		7.72	155	354
		8.05	151	371
		8.03	157	389
Mean		7.82	149	372
SD		0.219	7.76	13.6
%CV		2.8	5.2	3.7
%Bias		-10.5	-3.2	-7.7
N		5	5	5

Table 61. Long-Term Matrix Stability for PFHS in Human Serum

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC 6.15 ppb 55 Days	Medium QC 152 ppb 55 Days	High QC 402 ppb 55 Days
21-Aug-2000	18	7.01	163	387
		7.06	158	414
		7.10	169	375
		6.57	161	404
		6.56	168	435
Mean		6.86	164	403
SD		0.271	4.66	23.4
%CV		4.0	2.8	5.8
%Bias		11.5	7.9	0.2
N		5	5	5

Table 62. Long-Term Matrix Stability for M556 in Human Serum

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC 5.80 ppb 55 Days	Medium QC 152 ppb 55 Days	High QC 402 ppb 55 Days
21-Aug-2000	18	6.07	141	355
		5.67	137	382
		*4.60	149	340
		4.97	137	362
		5.79	143	394
Mean		5.42	141	367
SD		0.612	4.98	21.5
%CV		11.3	3.5	5.9
%Bias		-6.6	-7.2	-8.7
N		5	5	5

* > ± 20% deviation from theoretical

Table 63. Long-Term Matrix Stability for M570 in Human Serum

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC 8.60 ppb 55 Days	Medium QC 155 ppb 55 Days	High QC 405 ppb 55 Days
21-Aug-2000	18	7.93	147	373
		8.07	147	391
		7.87	158	373
		8.04	144	384
		8.20	150	408
Mean		8.02	149	386
SD		0.128	5.36	14.6
%CV		1.6	3.6	3.8
%Bias		-6.7	-3.9	-4.7
N		5	5	5

Table 64. PFOS Extraction Efficiency from Human Serum

Run 22

Low Concentration = 4.00 ng/mL

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		0.0679	14.7
		0.0683	14.6
		0.0735	13.6
	Mean	0.0699	14.3
2. Analyte prior and IS after extraction		0.0656	
		0.0605	
		0.0649	
	Mean	0.0637	
3. IS prior and Analyte after extraction			0.775
			0.726
			0.805
	Mean		0.769

Adjusted mean extraction efficiency = 44.4%
Mean extraction efficiency for the internal standard = 5.38%

High Concentration = 400 ng/mL

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		1.65	0.605
		1.55	0.643
		1.65	0.607
	Mean	1.62	0.618
2. Analyte prior and IS after extraction		0.522	
		0.538	
		0.518	
	Mean	0.526	
3. IS prior and Analyte after extraction			0.0396
			0.0379
			0.0401
	Mean		0.0392

Adjusted mean extraction efficiency = 30.1%
Mean extraction efficiency for the internal standard = 6.34%

Overall mean extraction efficiency for analyte = 37.3%
Overall mean extraction efficiency for internal standard = 5.86%

* Due to rounding, calculations based on displayed values may differ from actual.

Table 65. PFOSA Extraction Efficiency from Human Serum

Run 22

Low Concentration = 4.00 ng/mL

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		0.0275	36.3
		0.0222	45.0
		0.0214	46.8
	Mean	0.0237	42.7
2. Analyte prior and IS after extraction		0.0171	
		0.0147	
		0.0172	
	Mean	0.0163	
3. IS prior and Analyte after extraction			2.47
			2.25
			2.45
	Mean		2.39

Mean extraction efficiency for the internal standard = 5.60%

High Concentration = 400 ng/mL

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		2.58	0.387
		2.47	0.405
		2.65	0.377
	Mean	2.57	0.390
2. Analyte prior and IS after extraction		1.80	
		1.87	
		1.75	
	Mean	1.81	
3. IS prior and Analyte after extraction			0.0243
			0.0223
			0.0233
	Mean		0.0233

Mean extraction efficiency for the analyte = 70.4%

Overall mean extraction efficiency for analyte = 69.6%
Overall mean extraction efficiency for internal standard = 5.79%

* Due to rounding, calculations based on displayed values may differ from actual.

Table 66. PFOSAA Extraction Efficiency from Human Serum

Run 22

Low Concentration = 4.00 ng/mL

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		0.0246	40.6
		0.0257	39.0
		0.0238	42.0
	Mean	0.0247	40.5
2. Analyte prior and IS after extraction		0.0206	
		0.0186	
		0.0194	
	Mean	0.0195	
3. IS prior and Analyte after extraction			2.71
			2.45
			2.58
	Mean		2.58

Adjusted mean extraction efficiency = 62.6%
Mean extraction efficiency for the internal standard = 6.37%

High Concentration = 400 ng/mL

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		1.50	0.665
		1.44	0.693
		1.56	0.641
	Mean	1.50	0.666
2. Analyte prior and IS after extraction		0.962	
		0.959	
		0.917	
	Mean	0.946	
3. IS prior and Analyte after extraction			0.0419
			0.0394
			0.0413
	Mean		0.0409

Adjusted mean extraction efficiency = 62.7%
Mean extraction efficiency for the internal standard = 6.14%

Overall mean extraction efficiency for analyte = 62.7%
Overall mean extraction efficiency for internal standard = 6.26%

* Due to rounding, calculations based on displayed values may differ from actual.

Table 67. POAA Extraction Efficiency from Human Serum

Run 22

Low Concentration = 4.00 ng/mL

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		0.0288	34.7
		0.0291	34.3
		0.0302	33.1
	Mean	0.0294	34.0
2. Analyte prior and IS after extraction		0.00934	
		0.00760	
		0.0101	
	Mean	0.00901	
3. IS prior and Analyte after extraction			2.02
			1.93
			2.05
	Mean		2.00

Adjusted mean extraction efficiency = 16.8%
Mean extraction efficiency for the internal standard = 5.88%

High Concentration = 400 ng/mL

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		2.61	0.383
		2.59	0.386
		2.72	0.367
	Mean	2.64	0.379
2. Analyte prior and IS after extraction		0.377	
		0.385	
		0.383	
	Mean	0.382	
3. IS prior and Analyte after extraction			0.0248
			0.0227
			0.0241
	Mean		0.0239

Adjusted mean extraction efficiency = 14.3%
Mean extraction efficiency for the internal standard = 6.31%

Overall mean extraction efficiency for analyte = 15.6%
Overall mean extraction efficiency for internal standard = 6.10%

* Due to rounding, calculations based on displayed values may differ from actual.

Table 68. PFHS Extraction Efficiency from Human Serum

Run 22

Low Concentration = 4.00 ng/mL

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		0.0367	27.3
		0.0361	27.7
		0.0366	27.3
	Mean	0.0365	27.4
2. Analyte prior and IS after extraction		0.00870	
		0.00778	
		0.00878	
	Mean	0.00842	
3. IS prior and Analyte after extraction			1.72
			1.63
			1.71
	Mean		1.69

Adjusted mean extraction efficiency = 16.3%
Mean extraction efficiency for the internal standard = 6.17%

High Concentration = 400 ng/mL

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		3.49	0.286
		3.29	0.304
		3.58	0.279
	Mean	3.45	0.290
2. Analyte prior and IS after extraction		0.486	
		0.514	
		0.485	
	Mean	0.495	
3. IS prior and Analyte after extraction			0.0192
			0.0181
			0.0192
	Mean		0.0188

Adjusted mean extraction efficiency = 14.3%
Mean extraction efficiency for the internal standard = 6.48%

Overall mean extraction efficiency for analyte = 15.3%
Overall mean extraction efficiency for internal standard = 6.33%

* Due to rounding, calculations based on displayed values may differ from actual.

Table 69. M556 Extraction Efficiency from Human Serum

Run 22

Low Concentration = 4.00 ng/mL

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		0.00786	127
		0.00750	133
		0.00810	123
	Mean	0.00782	128
2. Analyte prior and IS after extraction		0.00421	
		0.00366	
		0.00310	
	Mean	0.00366	
3. IS prior and Analyte after extraction			7.54
			6.18
			6.78
	Mean		6.83

Mean extraction efficiency for the analyte = 46.8%
Adjusted mean extraction efficiency = 37.7%

High Concentration = 400 ng/mL

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		0.788	1.27
		0.758	1.32
		0.836	1.20
	Mean	0.794	1.26
2. Analyte prior and IS after extraction		0.337	
		0.326	
		0.303	
	Mean	0.322	
3. IS prior and Analyte after extraction			0.0753
			0.0690
			0.0709
	Mean		0.0717

Mean extraction efficiency for the analyte = 40.6%
Adjusted mean extraction efficiency = 40.4%

Overall mean extraction efficiency for analyte = 39.1%
Overall mean extraction efficiency for internal standard = 5.52%

* Due to rounding, calculations based on displayed values may differ from actual.

Table 70. M570 Extraction Efficiency from Human Serum

Run 22

Low Concentration = 4.00 ng/mL

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		0.0333	30.1
		0.0317	31.5
		0.0335	29.9
	Mean	0.0328	30.5
2. Analyte prior and IS after extraction		0.0244	
		0.0220	
		0.0244	
	Mean	0.0236	
3. IS prior and Analyte after extraction			1.92
			1.72
			1.74
	Mean		1.79

Adjusted mean extraction efficiency = 54.4%
Mean extraction efficiency for the internal standard = 5.87%

High Concentration = 400 ng/mL

Time of Spiking		Ratio Analyte/IS	Ratio IS/Analyte
1. Analyte and IS after extraction		2.20	0.454
		2.16	0.463
		2.35	0.426
	Mean	2.24	0.448
2. Analyte prior and IS after extraction		1.34	
		1.37	
		1.32	
	Mean	1.34	
3. IS prior and Analyte after extraction			0.0294
			0.0270
			0.0280
	Mean		0.0281

Adjusted mean extraction efficiency = 59.7%
Mean extraction efficiency for the internal standard = 6.27%

Overall mean extraction efficiency for analyte = 57.1%
Overall mean extraction efficiency for internal standard = 6.07%

* Due to rounding, calculations based on displayed values may differ from actual.

Table 71. Summary of Calibration Curve Parameters for PFOS in Human Plasma

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
12-Oct-2000	S00091-2	-0.000007	0.030663	-0.005620	0.9986	3.94	414
27-Oct-2000	PFOSMD-40	0.000000	0.028132	0.010586	0.9931	3.94	414

A, B, and C are coefficients used to define the calibration curve.

Table 72. Summary of Calibration Curve Parameters for PFOSA in Human Plasma

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
12-Oct-2000	S00091-2	-0.000011	0.073810	0.006421	0.9970	1.00	500
27-Oct-2000	PFOSMD-40	0.000001	0.052188	0.010966	0.9912	1.00	500

A, B, and C are coefficients used to define the calibration curve.

Table 73. Summary of Calibration Curve Parameters for PFOSAA in Human Plasma

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
12-Oct-2000	S00091-2	-0.000003	0.032601	-0.006279	0.9988	1.60	501
27-Oct-2000	PFOSMD-40	0.000005	0.021209	-0.002450	0.9873	1.60	501

A, B, and C are coefficients used to define the calibration curve.

Table 74. Summary of Calibration Curve Parameters for POAA in Human Plasma

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
12-Oct-2000	S00091-2	0.000000	0.017245	0.001072	0.9985	1.92	481
27-Oct-2000	PFOSMD-40	0.000002	0.014346	0.002192	0.9918	1.92	481

A, B, and C are coefficients used to define the calibration curve.

Table 75. Summary of Calibration Curve Parameters for PFHS in Human Plasma

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
12-Oct-2000	S00091-2	-0.000005	0.025818	-0.000970	0.9983	1.36	523
27-Oct-2000	PFOSMD-40	-0.000001	0.025534	0.011247	0.9953	1.36	523

A, B, and C are coefficients used to define the calibration curve.

Table 76. Summary of Calibration Curve Parameters for M556 in Human Plasma

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
12-Oct-2000	S00091-2	-0.000002	0.018315	0.005561	0.9954	2.50	500
31-Oct-2000	24	-0.000001	0.018373	0.001923	0.9972	2.50	500

A, B, and C are coefficients used to define the calibration curve.

Table 77. Summary of Calibration Curve Parameters for M570 in Human Plasma

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
12-Oct-2000	S00091-2	-0.000006	0.048518	0.005367	0.9978	1.00	500
27-Oct-2000	PFOSMD-40	0.000001	0.034031	0.011681	0.9910	1.00	500

A, B, and C are coefficients used to define the calibration curve.

Table 78. Back-Calculated Concentrations of Calibration Standards for PFOS in Human Plasma

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	3.94	5.17	11.3	23.6	44.2	85.4	209	332	414
12-Oct-2000	S00091-2	4.00	5.01	11.2	25.0	42.9	83.7	203	335	390
		3.86	5.35	11.1	24.3	43.5	85.3	209	350	424
27-Oct-2000	PFOSMD-40	3.72	5.18	11.3	19.4	45.5	82.1	214	337	369
		3.90	5.67	11.7	24.1	45.9	84.4	221	367	407

Table 79. Back-Calculated Concentrations of Calibration Standards for PFOSA in Human Plasma

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	1.00	2.50	10.0	25.0	50.0	100	250	400	500
12-Oct-2000	S00091-2	1.04	2.60	10.4	25.1	49.1	95.9	252	380	462
		0.941	2.53	8.93	25.7	50.5	102	255	444	508
27-Oct-2000	PFOSMD-40	0.961	2.59	9.81	20.2	53.4	103	259	356	443
		0.999	2.71	10.4	22.1	52.3	101	267	458	515

Table 80. Back-Calculated Concentrations of Calibration Standards for PFOSAA in Human Plasma

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	1.60	3.10	10.6	25.6	50.6	101	251	401	501
12-Oct-2000	S00091-2	1.65	3.12	10.7	25.3	50.5	98.2	255	393	469
		1.54	3.10	10.4	26.3	51.0	101	245	432	513
27-Oct-2000	PFOSMD-40	1.66	3.50	11.7	21.3	53.1	104	299	406	440
		1.47	2.96	10.8	23.5	47.3	90.3	261	441	475

Table 81. Back-Calculated Concentrations of Calibration Standards for POAA in Human Plasma

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	1.92	3.36	10.6	25.0	49.0	97.1	241	385	481
12-Oct-2000	S00091-2	2.00	3.24	10.5	26.1	49.3	97.5	241	386	477
		1.96	3.11	10.1	25.2	52.1	95.0	239	392	477
27-Oct-2000	PFOSMD-40	1.73	3.01	10.5	22.9	49.7	99.1	250	367	439
		2.30	3.12	10.9	25.1	50.7	96.7	262	422	474

Table 82. Back-Calculated Concentrations of Calibration Standards for PFHS in Human Plasma

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	1.36	2.92	10.7	26.5	52.6	104	262	418	523
12-Oct-2000	S00091-2	1.28	2.74	10.6	27.5	50.2	104	253	441	518
		1.44	3.08	10.8	26.9	53.2	104	259	413	524
27-Oct-2000	PFOSMD-40	1.52	2.72	10.6	24.2	54.6	107	259	466	489
		1.28	2.78	10.4	28.4	54.1	102	270	425	491

Table 83. Back-Calculated Concentrations of Calibration Standards for M556 in Human Plasma

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	2.50	10.0	25.0	50.0	100	250	400	500
12-Oct-2000	S00091-2	2.48	11.3	27.9	49.7	101	259	398	521
		2.47	9.43	24.5	43.7	96.7	245	384	499
31-Oct-2000	24	2.33	9.67	24.3	51.9	95.2	255	388	499
		2.70	9.81	25.8	49.3	104	249	438	470

Table 84. Back-Calculated Concentrations of Calibration Standards for M570 in Human Plasma

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	1.00	2.50	10.0	25.0	50.0	100	250	400	500
12-Oct-2000	S00091-2	0.991	2.57	10.2	25.0	50.8	97.0	250	380	451
		1.00	2.48	9.46	25.4	50.3	101	244	448	528
27-Oct-2000	PFOSMD-40	0.993	2.71	10.6	21.2	53.6	104	284	401	444
		0.951	2.65	10.4	20.9	48.6	93.5	254	443	481

Table 85. Lower Limit of Quantitation for PFOS in Human Plasma

All concentrations are expressed as ppb.

Run Date	Run Number	LLOQ (3.94 ppb)
27-Oct-2000	PFOS MD 40	3.98
		3.51
		3.89
		4.04
		4.06
		3.77
Mean		3.88
S.D.		0.208
%CV		5.4
%Theoretical		98.5
%Bias		-1.5
N		6

Table 86. Lower Limit of Quantitation for PFOSA in Human Plasma

All concentrations are expressed as ppb.

Run Date	Run Number	LLOQ (1.00 ppb)
27-Oct-2000	PFOS MD 40	1.08
		1.07
		1.11
		0.900
		1.01
		0.865
Mean		1.01
S.D.		0.102
%CV		10.1
%Theoretical		101.0
%Bias		1.0
N		6

Table 87. Lower Limit of Quantitation for PFOSAA in Human Plasma

All concentrations are expressed as ppb.

Run Date	Run Number	LLOQ (1.60 ppb)
27-Oct-2000	PFOS MD 40	1.63
		1.58
		1.54
		1.39
		1.63
		1.57
Mean		1.56
S.D.		0.0889
%CV		5.7
%Theoretical		97.5
%Bias		-2.5
N		6

Table 88. Lower Limit of Quantitation for POAA in Human Plasma

All concentrations are expressed as ppb.

Run Date	Run Number	LLOQ (1.92 ppb)
27-Oct-2000	PFOS MD 40	1.72
		1.84
		1.80
		1.74
		1.79
		1.62
Mean		1.75
S.D.		0.0776
%CV		4.4
%Theoretical		91.1
%Bias		-8.9
N		6

Table 89. Lower Limit of Quantitation for PFHS in Human Plasma

All concentrations are expressed as ppb.

Run Date	Run Number	LLOQ (1.36 ppb)
27-Oct-2000	PFOS MD 40	1.23
		1.29
		1.36
		1.35
		*1.06
		1.42
Mean		1.29
S.D.		0.128
%CV		9.9
%Theoretical		94.9
%Bias		-5.1
N		6

* > ± 20% deviation from theoretical

Table 90. Lower Limit of Quantitation for M556 in Human Plasma

All concentrations are expressed as ppb.

Run Date	Run Number	LLOQ (2.50 ppb)
31-Oct-2000	24	2.44
		2.25
		2.50
		2.65
		2.50
		2.34
Mean		2.45
S.D.		0.139
%CV		5.7
%Theoretical		98.0
%Bias		-2.0
N		6

Table 91. Lower Limit of Quantitation for M570 in Human Plasma

All concentrations are expressed as ppb.

Run Date	Run Number	LLOQ (1.00 ppb)
27-Oct-2000	PFOS MD 40	1.14
		1.02
		1.01
		1.01
		1.01
		0.997
Mean		1.03
S.D.		0.0538
%CV		5.2
%Theoretical		103.0
%Bias		3.0
N		6

Table 92. Intra-Assay Precision for PFOS Quality Controls in Human Plasma

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC 6.40 ppb	Medium QC 126 ppb	High QC 332 ppb
12-Oct-2000	S00091-2	7.33	132	343
		7.38	129	331
		*7.89	138	334
		7.07	132	334
		6.98	137	340
Mean		7.19	134	336
S.D.		0.195	3.78	4.93
%CV		2.7	2.8	1.5
%Theoretical		112.3	106.3	101.2
%Bias		12.3	6.3	1.2
n		4	5	5

* Sample deactivated due to unacceptable internal standard response.

Table 93. Intra-Assay Precision for PFOSA Quality Controls in Human Plasma

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC 4.00 ppb	Medium QC 150 ppb	High QC 400 ppb
12-Oct-2000	S00091-2	4.69	148	423
		4.73	157	409
		*4.67	172	425
		4.53	166	414
		4.66	163	409
Mean		4.65	161	416
S.D.		0.0866	9.15	7.62
%CV		1.9	5.7	1.8
%Theoretical		116.3	107.3	104.0
%Bias		16.3	7.3	4.0
n		4	5	5

* Sample deactivated due to unacceptable internal standard response.

Table 94. Intra-Assay Precision for PFOSAA Quality Controls in Human Plasma

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC 4.60 ppb	Medium QC 151 ppb	High QC 401 ppb
12-Oct-2000	S00091-2	5.20	146	411
		5.50	148	423
		*7.43	169	431
		4.96	156	404
		4.89	155	425
Mean		5.14	155	419
S.D.		0.276	9.04	11.0
%CV		5.4	5.8	2.6
%Theoretical		111.7	102.6	104.5
%Bias		11.7	2.6	4.5
n		4	5	5

* Sample deactivated due to unacceptable internal standard response.

Table 95. Intra-Assay Precision for POAA Quality Controls in Human Plasma

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC 4.81 ppb	Medium QC 145 ppb	High QC 385 ppb
12-Oct-2000	S00091-2	5.33	144	400
		5.17	157	395
		*7.82	163	399
		4.86	155	394
		4.69	152	401
Mean		5.01	154	398
S.D.		0.290	6.98	3.11
%CV		5.8	4.5	0.8
%Theoretical		104.2	106.2	103.4
%Bias		4.2	6.2	3.4
n		4	5	5

* Sample deactivated due to unacceptable internal standard response.

Table 96. Intra-Assay Precision for PFHS Quality Controls in Human Plasma

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC 4.48 ppb	Medium QC 156 ppb	High QC 417 ppb
12-Oct-2000	S00091-2	4.91	162	406
		4.80	158	388
		*5.41	162	399
		4.72	150	403
		4.58	158	401
Mean		4.75	158	399
S.D.		0.139	4.90	6.88
%CV		2.9	3.1	1.7
%Theoretical		106.0	101.3	95.7
%Bias		6.0	1.3	-4.3
n		4	5	5

* Sample deactivated due to unacceptable internal standard response.

Table 97. Intra-Assay Precision for M556 Quality Controls in Human Plasma

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC 4.00 ppb	Medium QC 150 ppb	High QC 400 ppb
12-Oct-2000	S00091-2	4.65	147	441
		4.35	140	**492
		*4.76	150	459
		4.14	146	403
		3.89	159	431
Mean		4.26	148	445
S.D.		0.322	6.95	33.1
%CV		7.6	4.7	7.4
%Theoretical		106.5	98.7	111.3
%Bias		6.5	-1.3	11.3
n		4	5	5

* Sample deactivated due to unacceptable internal standard response.
** > ±20% deviation from theoretical

Table 98. Intra-Assay Precision for M570 Quality Controls in Human Plasma

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC 4.00 ppb	Medium QC 150 ppb	High QC 400 ppb
12-Oct-2000	S00091-2	**4.82	146	407
		4.75	161	407
		*5.89	170	414
		4.56	165	393
		4.74	158	403
Mean		4.72	160	405
S.D.		0.111	9.03	7.69
%CV		2.4	5.6	1.9
%Theoretical		118.0	106.7	101.3
%Bias		18.0	6.7	1.3
n		4	5	5

* Sample deactivated due to unacceptable internal standard response.
** > ±20% deviation from theoretical

Table 99. Summary of Calibration Curve Parameters for PFOS in Serum Diluted with Buffer

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
23-Oct-2000	PFOSMD-39	-0.000001	0.009444	-0.004315	0.9954	5.30	504

A, B, and C are coefficients used to define the calibration curve.

Table 100. Summary of Calibration Curve Parameters for PFOSA in Serum Diluted with Buffer

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
23-Oct-2000	PFOSMD-39	0.000000	0.014752	0.002265	0.9946	1.00	500

A, B, and C are coefficients used to define the calibration curve.

Table 101. Summary of Calibration Curve Parameters for PFOSAA in Serum Diluted with Buffer

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
23-Oct-2000	PFOSMD-39	0.000002	0.009880	0.004993	0.9960	1.00	500

A, B, and C are coefficients used to define the calibration curve.

Table 102. Summary of Calibration Curve Parameters for POAA in Serum Diluted with Buffer

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
23-Oct-2000	PFOSMD-39	-0.000001	0.011351	-0.000137	0.9967	1.70	501

A, B, and C are coefficients used to define the calibration curve.

Table 103. Summary of Calibration Curve Parameters for PFHS in Serum Diluted with Buffer

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
23-Oct-2000	PFOSMD-39	-0.000003	0.018220	0.007453	0.9980	1.00	500

A, B, and C are coefficients used to define the calibration curve.

Table 104. Summary of Calibration Curve Parameters for M556 in Serum Diluted with Buffer

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
23-Oct-2000	PFOSMD-39	0.000000	0.008729	-0.000146	0.9936	1.00	500

A, B, and C are coefficients used to define the calibration curve.

Table 105. Summary of Calibration Curve Parameters for M570 in Serum Diluted with Buffer

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	A	B	C	R-Squared	LLOQ	ULOQ
23-Oct-2000	PFOSMD-39	0.000000	0.015628	0.005711	0.9969	1.00	500

A, B, and C are coefficients used to define the calibration curve.

Table 106. Back-Calculated Concentrations of Calibration Standards for PFOS in Serum Diluted with Buffer

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	5.30	6.80	14.3	29.3	54.3	104	254	404	504
23-Oct-2000	PFOSMD-39	5.32	6.18	14.0	25.6	52.3	102	264	410	476
		5.45	7.23	14.8	30.1	54.6	113	265	417	488

Table 107. Back-Calculated Concentrations of Calibration Standards for PFOSA in Serum Diluted with Buffer

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	1.00	2.50	10.0	25.0	50.0	100	250	400	500
23-Oct-2000	PFOSMD-39	1.03	2.21	9.29	22.6	50.2	98.2	265	410	455
		1.03	2.45	10.1	25.8	50.6	112	271	423	468

Table 108. Back-Calculated Concentrations of Calibration Standards for PFOSAA in Serum Diluted with Buffer

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	1.00	2.50	10.0	25.0	50.0	100	250	400	500
23-Oct-2000	PFOSMD-39	1.12	2.50	9.73	23.6	50.0	104	267	408	485
		0.901	2.41	9.41	24.1	50.6	111	254	399	479

Table 109. Back-Calculated Concentrations of Calibration Standards for POAA in Serum Diluted with Buffer

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	1.70	3.20	10.7	25.7	50.7	101	254	401	501
23-Oct-2000	PFOSMD-39	1.56	3.22	10.4	24.7	50.9	99.8	260	401	458
		1.88	3.10	10.6	25.3	50.8	109	263	432	491

Table 110. Back-Calculated Concentrations of Calibration Standards for PFHS in Serum Diluted with Buffer

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	1.00	2.50	10.0	25.0	50.0	100	250	400	500
23-Oct-2000	PFOSMD-39	1.01	2.40	10.1	24.2	49.6	99.3	254	424	450
		1.01	2.53	10.0	24.3	49.0	106	260	433	473

Table 111. Back-Calculated Concentrations of Calibration Standards for M556 in Serum Diluted with Buffer

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	2.50	10.0	25.0	50.0	100	250	400	500
23-Oct-2000	PFOSMD-39	2.68	10.6	22.9	51.3	109	283	432	505
		2.36	9.33	22.9	48.6	102	242	379	451

Table 112. Back-Calculated Concentrations of Calibration Standards for M570 in Serum Diluted with Buffer

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	1.00	2.50	10.0	25.0	50.0	100	250	400	500
23-Oct-2000	PFOSMD-39	1.07	2.48	9.93	22.5	51.6	103	268	408	489
		0.964	2.34	9.59	24.6	50.8	110	254	393	476

Table 113. PFOS Quality Controls Samples Prepared in Serum Diluted with Buffer

Run Date	Run Number	193 ng/mL DF=10	443 ng/mL DF=10	4040 ng/mL DF=10	4040 ng/mL DF=50
23-Oct-2000	PFOSMD-39	204	464	4240	*3180
		219	423	4420	*3020
		205	451	4320	*3150
		202	477	4320	*3100
Mean		208	454	4330	3110
S.D.		7.77	23.1	73.7	69.9
%CV		3.7	5.1	1.7	2.2
%Theoretical		107.8	102.5	107.2	77.0
%Bias		7.8	2.5	7.2	-23.0
n		4	4	4	4

* >±20% deviation from theoretical

Table 114. PFOSA Quality Control Samples Prepared in Serum Diluted with Buffer

Run Date	Run Number	150 ng/mL DF=10	400 ng/mL DF=10	4000 ng/mL DF=10	4000 ng/mL DF=50
23-Oct-2000	PFOSMD-39	147	388	4020	*2380
		147	360	3850	*2240
		145	370	3980	*2210
		136	409	3960	*2210
Mean		144	382	3950	2260
S.D.		5.25	21.5	72.7	81.2
%CV		3.6	5.6	1.8	3.6
%Theoretical		96.0	95.5	98.8	56.5
%Bias		-4.0	-4.5	-1.3	-43.5
n		4	4	4	4

* >±20% deviation from theoretical

Table 115. PFOSAA Quality Control Samples Prepared in Serum Diluted with Buffer

Run Date	Run Number	153 ng/mL DF=10	403 ng/mL DF=10	4000 ng/mL DF=10	4000 ng/mL DF=50
23-Oct-2000	PFOSMD-39	144	405	3920	*2750
		146	379	4100	*2560
		142	389	3970	*2620
		142	417	4010	*2550
Mean		144	398	4000	2620
S.D.		1.91	16.8	76.2	92.0
%CV		1.3	4.2	1.9	3.5
%Theoretical		94.1	98.8	100.0	65.5
%Bias		-5.9	-1.2	0.0	-34.5
n		4	4	4	4

* >±20% deviation from theoretical

Table 116. POAA Quality Control Samples Prepared in Serum Diluted with Buffer

Run Date	Run Number	157 ng/mL DF=10	407 ng/mL DF=10	4010 ng/mL DF=10	4010 ng/mL DF=50
23-Oct-2000	PFOSMD-39	163	410	4230	3610
		174	387	4190	3310
		164	418	4280	3330
		166	411	4140	3350
Mean		167	407	4210	3400
S.D.		4.99	13.5	59.4	141
%CV		3.0	3.3	1.4	4.1
%Theoretical		106.4	100.0	105.0	84.8
%Bias		6.4	0.0	5.0	-15.2
n		4	4	4	4

Table 117. PFHS Quality Control Samples Prepared in Serum Diluted with Buffer

Run Date	Run Number	154 ng/mL DF=10	404 ng/mL DF=10	4000 ng/mL DF=10	4000 ng/mL DF=50
23-Oct-2000	PFOSMD-39	157	421	4200	3490
		163	389	4210	3300
		161	416	4330	3410
		166	419	4300	3260
Mean		162	411	4260	3370
S.D.		3.77	15.0	64.8	105
%CV		2.3	3.6	1.5	3.1
%Theoretical		105.2	101.7	106.5	84.3
%Bias		5.2	1.7	6.5	-15.8
n		4	4	4	4

Table 118. M556 Quality Control Samples Prepared in Serum Diluted with Buffer

Run Date	Run Number	151 ng/mL DF=10	401 ng/mL DF=10	4000 ng/mL DF=10	4000 ng/mL DF=50
23-Oct-2000	PFOSMD-39	156	421	4480	*2980
		157	385	4600	*2740
		145	403	4710	*2780
		148	417	4490	*2710
Mean		152	407	4570	2800
S.D.		5.92	16.3	108	122
%CV		3.9	4.0	2.4	4.4
%Theoretical		100.7	101.5	114.3	70.0
%Bias		0.7	1.5	14.3	-30.0
n		4	4	4	4

* >±20% deviation from theoretical

Table 119. M570 Quality Control Samples Prepared in Serum Diluted with Buffer

Run Date	Run Number	157 ng/mL DF=10	407 ng/mL DF=10	4010 ng/mL DF=10	4010 ng/mL DF=50
23-Oct-2000	PFOSMD-39	159	430	4240	*2910
		167	401	4390	*2730
		158	408	4290	*2700
		160	441	4330	*2680
Mean		161	420	4310	2760
S.D.		4.08	18.7	63.4	105
%CV		2.5	4.5	1.5	3.8
%Theoretical		102.5	103.2	107.5	68.8
%Bias		2.5	3.2	7.5	-31.2
n		4	4	4	4

* >±20% deviation from theoretical

Table 120. Summary of Calibration Curve Parameters for PFOS (Serum vs. Plasma)

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	Matrix	A	B	C	R-Squared	LLOQ	ULOQ
27-Oct-2000	PFOSMD-40	Plasma	0.000000	0.028132	0.010586	0.9931	3.94	414
27-Oct-2000	PFOSMD-46	Serum	-0.000003	0.031081	0.084427	0.9506	36.8	454
25-Apr-2001	25	Plasma	-0.000003	0.048192	0.045435	0.9841	3.28	413
25-Apr-2001	26	Serum	-0.000020	0.057680	-0.301033	0.9934	36.8	454

A, B, and C are coefficients used to define the calibration curve.

Table 121. Summary of Calibration Curve Parameters for PFOSA (Serum vs. Plasma)

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	Matrix	A	B	C	R-Squared	LLOQ	ULOQ
25-Apr-2001	25	Plasma	-0.000005	0.120662	0.028023	0.9887	1.00	500
25-Apr-2001	26	Serum	-0.000032	0.166445	0.051786	0.9873	1.00	502

A, B, and C are coefficients used to define the calibration curve.

Table 122. Summary of Calibration Curve Parameters for PFOSAA (Serum vs. Plasma)

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	Matrix	A	B	C	R-Squared	LLOQ	ULOQ
25-Apr-2001	25	Plasma	0.000009	0.033608	0.035470	0.9938	1.30	500
25-Apr-2001	26	Serum	0.000007	0.044621	-0.040847	0.9947	4.20	503

A, B, and C are coefficients used to define the calibration curve.

Table 123. Summary of Calibration Curve Parameters for POAA (Serum vs. Plasma)

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	Matrix	A	B	C	R-Squared	LLOQ	ULOQ
27-Oct-2000	PFOSMD-40	Plasma	0.000002	0.014346	0.002192	0.9918	1.92	481
27-Oct-2000	PFOSMD-46	Serum	0.000003	0.016839	0.003246	0.9894	6.82	461
25-Apr-2001	25	Plasma	-0.000003	0.011215	0.005552	0.9929	1.44	481
25-Apr-2001	26	Serum	0.000001	0.011414	0.000898	0.9931	6.82	461

A, B, and C are coefficients used to define the calibration curve.

Table 124. Summary of Calibration Curve Parameters for PFHS (Serum vs. Plasma)

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	Matrix	A	B	C	R-Squared	LLOQ	ULOQ
27-Oct-2000	PFOSMD-40	Plasma	-0.000001	0.025534	0.011247	0.9953	1.36	523
27-Oct-2000	PFOSMD-46	Serum	-0.000012	0.033256	0.018733	0.9890	3.85	431

A, B, and C are coefficients used to define the calibration curve.

Table 125. Summary of Calibration Curve Parameters for M556 (Serum vs. Plasma)

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	Matrix	A	B	C	R-Squared	LLOQ	ULOQ
27-Oct-2000	PFOSMD-40	Plasma	0.000002	0.016888	0.007835	0.9949	2.50	500
27-Oct-2000	PFOSMD-46	Serum	0.000005	0.014650	0.008960	0.9903	3.80	501

A, B, and C are coefficients used to define the calibration curve.

Table 126. Summary of Calibration Curve Parameters for M570 (Serum vs. Plasma)

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	Matrix	A	B	C	R-Squared	LLOQ	ULOQ
27-Oct-2000	PFOSMD-40	Plasma	0.000001	0.034031	0.011681	0.9910	1.00	500
27-Oct-2000	PFOSMD-46	Serum	0.000003	0.034699	0.028660	0.9919	5.20	503
25-Apr-2001	25	Plasma	0.000009	0.039312	0.045599	0.9964	1.00	500
25-Apr-2001	26	Serum	0.000002	0.046817	0.021413	0.9931	3.70	503

A, B, and C are coefficients used to define the calibration curve.

Table 127. Back-Calculated Concentrations of Calibration Standards for PFOS (Serum vs. Plasma)

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	**Run Number**	**Matrix**	**3.94**	**5.17**	**11.3**	**23.6**	**44.2**	**85.4**	**209**	**332**	**414**
27-Oct-2000	PFOSMD-40	Plasma	3.72	5.18	11.3	19.4	45.5	82.1	214	337	369
			3.90	5.67	11.7	24.1	45.9	84.4	221	367	407
Run Date	**Run Number**	**Matrix**	**36.8**	**38.0**	**44.3**	**56.8**	**77.7**	**120**	**245**	**370**	**454**
27-Oct-2000	PFOSMD-46	Serum	34.0	31.7	37.3	49.3	70.7	114	247	374	392
			50.5	39.0	45.8	60.7	78.7	125	282	418	436
Run Date	**Run Number**	**Matrix**	**3.28**	**4.52**	**10.7**	**23.0**	**43.5**	**84.6**	**208**	**331**	**413**
25-Apr-2001	25	Plasma	2.70	3.90	9.65	21.4	42.2	80.3	190	324	392
			4.15	4.63	11.3	25.0	47.6	90.3	217	336	441
Run Date	**Run Number**	**Matrix**	**36.8**	**38.0**	**44.3**	**56.8**	**77.7**	**120**	**245**	**370**	**454**
25-Apr-2001	26	Serum	38.3	35.5	44.2	54.7	71.1	124	234	363	420
			37.4	39.0	46.1	56.1	75.9	128	256	400	459

Table 128. Back-Calculated Concentrations of Calibration Standards for PFOSA (Serum vs. Plasma)

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	Matrix	1.00	2.50	10.0	25.0	50.0	100	250	400	500
25-Apr-2001	25	Plasma	0.935	2.52	7.89	23.0	45.4	102	223	387	477
			*1.77	2.99	10.3	27.3	52.5	113	254	402	546
25-Apr-2001	26	Serum	0.951	1.96	9.62	25.6	48.1	109	240	403	422
			1.18	2.21	10.7	24.3	52.4	111	279	450	491

* Sample deactivated as an outlier

Table 129. Back-Calculated Concentrations of Calibration Standards for PFOSAA (Serum vs. Plasma)

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	Matrix	1.30	2.80	10.3	25.3	50.3	100	250	400	500
25-Apr-2001	25	Plasma	1.28	2.65	8.27	24.7	50.7	102	253	399	480
			*2.23	3.22	9.79	24.9	54.4	110	257	391	509
Run Date	**Run Number**	**Matrix**	**4.20**	**5.70**	**13.2**	**28.2**	**53.2**	**103**	**253**	**403**	**503**
25-Apr-2001	26	Serum	4.27	5.31	12.8	26.9	50.7	119	259	391	477
			4.38	5.78	13.6	25.0	51.9	111	260	407	511

* Sample deactivated as an outlier

Table 130. Back-Calculated Concentrations of Calibration Standards for POAA (Serum vs. Plasma)

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	Matrix	1.92	3.36	10.6	25.0	49.0	97.1	241	385	481
27-Oct-2000	PFOSMD-40	Plasma	1.73	3.01	10.5	22.9	49.7	99.1	250	367	439
			2.30	3.12	10.9	25.1	50.7	96.7	262	422	474
Run Date	**Run Number**	**Matrix**	**6.82**	**8.18**	**15.0**	**28.6**	**51.4**	**97.3**	**234**	**370**	**461**
27-Oct-2000	PFOSMD-46	Serum	7.32	6.86	13.9	27.2	51.9	105	252	399	421
			*18.3	9.23	14.9	29.4	46.2	94.9	246	392	424
Run Date	**Run Number**	**Matrix**	**1.44**	**2.88**	**10.1**	**24.5**	**48.5**	**96.6**	**241**	**385**	**481**
25-Apr-2001	25	Plasma	1.35	2.65	10.5	24.1	43.4	91.1	225	396	470
			*2.14	3.35	11.2	26.6	46.5	90.9	241	405	498
Run Date	**Run Number**	**Matrix**	**6.82**	**8.18**	**15.0**	**28.6**	**51.4**	**97.3**	**234**	**370**	**461**
25-Apr-2001	26	Serum	7.98	8.28	15.6	28.8	50.2	103	242	383	457
			6.23	7.23	15.0	26.9	48.0	100	235	378	433

* Sample deactivated as an outlier

Table 131. Back-Calculated Concentrations of Calibration Standards for PFHS (Serum vs. Plasma)

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	Matrix	1.36	2.92	10.7	26.5	52.6	104	262	418	523
27-Oct-2000	PFOSMD-40	Plasma	1.52✓	2.72	10.6	24.2	54.6	107	259	466	489
			1.28	2.78	10.4	28.4	54.1	102	270	425	491
Run Date	**Run Number**	**Matrix**	**3.85**	**5.13**	**11.5**	**24.4**	**45.7**	**88.9**	**217**	**345**	**431**
27-Oct-2000	PFOSMD-46	Serum	4.08	4.36	11.2	21.8	47.5	89.1	235	371	363
			*15.4	5.80	10.6	23.5	49.0	89.7	230	388	389

* Sample deactivated as an outlier

Table 132. Back-Calculated Concentrations of Calibration Standards for M556 (Serum vs. Plasma)

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	Matrix	2.50	10.0	25.0	50.0	100	250	400	500
27-Oct-2000	PFOSMD-40	Plasma	2.64	10.7	23.4	52.9	101	276	396	456
			2.35	9.95	23.3	48.3	94.1	260	440	481
Run Date	**Run Number**	**Matrix**	**3.80**	**11.3**	**26.3**	**51.3**	**101**	**251**	**401**	**501**
27-Oct-2000	PFOSMD-46	Serum	3.89	11.1	25.3	53.7	116	280	441	480
			3.91	10.0	25.7	42.9	102	258	396	450

Table 133. Back-Calculated Concentrations of Calibration Standards for M570 (Serum vs. Plasma)

Quadratic weighted $1/x^2$. All concentrations are expressed as ppb.

Run Date	Run Number	Matrix	1.00	2.50	10.0	25.0	50.0	100	250	400	500
27-Oct-2000	PFOSMD-40	Plasma	0.993	2.71	10.6	21.2	53.6	104	284	401	444
			0.951	2.65	10.4	20.9	48.6	93.5	254	443	481
Run Date	**Run Number**	**Matrix**	**3.70**	**5.20**	**12.7**	**27.7**	**52.7**	**103**	**253**	**403**	**503**
27-Oct-2000	PFOSMD-46	Serum	*5.54	4.92	11.8	27.4	51.0	116	277	432	464
			*16.4	5.75	12.4	26.9	45.5	106	271	404	468
Run Date	**Run Number**	**Matrix**	**1.00**	**2.50**	**10.0**	**25.0**	**50.0**	**100**	**250**	**400**	**500**
25-Apr-2001	25	Plasma	1.01	2.43	8.50	24.6	52.8	105	246	420	483
			*1.90	2.66	9.29	25.4	51.9	108	247	387	504
Run Date	**Run Number**	**Matrix**	**3.70**	**5.20**	**12.7**	**27.7**	**52.7**	**103**	**253**	**403**	**503**
25-Apr-2001	26	Serum	4.07	4.60	12.5	27.4	50.3	118	262	395	478
			3.94	4.60	13.3	26.0	49.7	109	264	405	502

* Sample deactivated as an outlier

Table 134. PFOS Quality Controls (Serum vs. Plasma)

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC (Plasma) 6.40 ppb	Medium QC (Plasma) 126 ppb	High QC (Plasma) 332 ppb
27-Oct-2000	PFOSMD-40	6.44	115	316
		5.99	136	326
		5.91	118	298
Mean		6.11	123	313
SD		0.286	11.4	14.2
%CV		4.7	9.3	4.5
%Bias		-4.5	-2.4	-5.7
n		3	3	3
Run Date	**Run Number**	**Low QC (Serum) 39.3 ppb**	**Medium QC (Serum) 161 ppb**	**High QC (Serum) 370 ppb**
27-Oct-2000	PFOSMD-46	35.0	164	336
		35.6	151	*199
		35.5	149	343
		35.8	152	346
Mean		35.5	154	342
SD		0.340	6.78	5.13
%CV		1.0	4.4	1.5
%Bias		-9.7	-4.3	-7.6
n		4	4	3

* Sample deactivated due to a sample preparation error.

** > ±20% deviation from theoretical

Table 134. PFOS Quality Controls (Serum vs. Plasma) Continued

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC (Plasma) 5.75 ppb	Medium QC (Plasma) 126 ppb	High QC (Plasma) 331 ppb
25-Apr-2001	25	5.44	143	333
		5.62	139	325
		5.59	136	347
		5.90	132	333
Mean		5.64	138	335
SD		0.192	4.65	9.15
%CV		3.4	3.4	2.7
%Bias		-1.9	9.5	1.2
n		4	4	4
Run Date	**Run Number**	**Low QC (Serum) 39.3 ppb**	**Medium QC (Serum) 161 ppb**	**High QC (Serum) 370 ppb**
25-Apr-2001	26	41.3	174	377
		45.4	182	388
		45.8	172	408
		45.6	169	381
Mean		44.5	174	389
SD		2.16	5.56	13.8
%CV		4.9	3.2	3.5
%Bias		13.2	8.1	5.1
n		4	4	4

** > ±20% deviation from theoretical

Table 135. PFOSA Quality Controls (Serum vs. Plasma)

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC (Plasma) 4.00 ppb	Medium QC (Plasma) 150 ppb	High QC (Plasma) 400 ppb
25-Apr-2001	25	4.06	158	395
		3.99	175	412
		4.49	170	438
		4.73	159	424
Mean		4.32	166	417
SD		0.353	8.35	18.2
%CV		8.2	5.0	4.4
%Bias		8.0	10.7	4.3
n		4	4	4
Run Date	**Run Number**	**Low QC (Serum) 4.00 ppb**	**Medium QC (Serum) 150 ppb**	**High QC (Serum) 400 ppb**
25-Apr-2001	26	3.52	153	390
		4.75	163	431
		4.55	155	417
		**4.85	151	377
Mean		4.42	156	404
SD		0.611	5.26	24.6
%CV		13.8	3.4	6.1
%Bias		10.5	4.0	1.0
n		4	4	4

* Sample deactivated due to a sample preparation error.

** > ±20% deviation from theoretical

Table 136. PFOSAA Quality Controls (Serum vs. Plasma)

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC (Plasma) 4.30 ppb	Medium QC (Plasma) 150 ppb	High QC (Plasma) 400 ppb
25-Apr-2001	25	3.59	**187	429
		3.85	175	412
		3.74	172	440
		4.96	169	429
Mean		4.04	176	428
SD		0.626	7.89	11.6
%CV		15.5	4.5	2.7
%Bias		-6.0	17.3	7.0
n		4	4	4
Run Date	**Run Number**	**Low QC (Serum) 7.20 ppb**	**Medium QC (Serum) 153 ppb**	**High QC (Serum) 403 ppb**
25-Apr-2001	26	6.74	150	363
		8.11	162	409
		8.29	159	396
		8.37	157	376
Mean		7.88	157	386
SD		0.766	5.10	20.5
%CV		9.7	3.2	5.3
%Bias		9.4	2.6	-4.2
n		4	4	4

* Sample deactivated due to a sample preparation error.

** > ±20% deviation from theoretical

Table 137. POAA Quality Controls (Serum vs. Plasma)

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC (Plasma) 4.81 ppb	Medium QC (Plasma) 145 ppb	High QC (Plasma) 385 ppb
27-Oct-2000	PFOSMD-40	4.57	146	373
		4.31	167	419
		4.52	147	393
Mean		4.47	153	395
SD		0.138	11.8	23.1
%CV		3.1	7.7	5.8
%Bias		-7.1	5.5	2.6
n		3	3	3
Run Date	**Run Number**	**Low QC (Serum) 9.54 ppb**	**Medium QC (Serum) 143 ppb**	**High QC (Serum) 370 ppb**
27-Oct-2000	PFOSMD-46	9.42	158	373
		9.17	146	*253
		8.53	148	375
		9.62	150	388
Mean		9.19	151	379
SD		0.474	5.26	8.14
%CV		5.2	3.5	2.1
%Bias		-3.7	5.6	2.4
n		4	4	3

* Sample deactivated due to a sample preparation error.

** > ±20% deviation from theoretical

Table 137. POAA Quality Controls (Serum vs. Plasma) Continued

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC (Plasma) 4.32 ppb	Medium QC (Plasma) 145 ppb	High QC (Plasma) 385 ppb
25-Apr-2001	25	5.13	143	394
		4.47	145	383
		4.35	145	412
		4.44	137	404
Mean		4.60	143	398
SD		0.359	3.79	12.6
%CV		7.8	2.7	3.2
%Bias		6.5	-1.4	3.4
n		4	4	4
Run Date	**Run Number**	**Low QC (Serum) 9.54 ppb**	**Medium QC (Serum) 143 ppb**	**High QC (Serum) 370 ppb**
25-Apr-2001	26	10.1	157	374
		11.4	152	389
		**13.2	149	385
		9.82	152	391
Mean		11.1	153	385
SD		1.54	3.32	7.59
%CV		13.9	2.2	2.0
%Bias		16.4	7.0	4.1
n		4	4	4

** > ±20% deviation from theoretical

Table 138. PFHS Quality Controls (Serum vs. Plasma)

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC (Plasma) 4.48 ppb	Medium QC (Plasma) 156 ppb	High QC (Plasma) 417 ppb
27-Oct-2000	PFOSMD-40	4.62	157	397
		4.35	173	407
		4.39	151	384
Mean		4.45	160	396
SD		0.146	11.4	11.5
%CV		3.3	7.1	2.9
%Bias		-0.7	2.6	-5.0
n		3	3	3
Run Date	**Run Number**	**Low QC (Serum) 6.41 ppb**	**Medium QC (Serum) 132 ppb**	**High QC (Serum) 345 ppb**
27-Oct-2000	PFOSMD-46	5.65	144	343
		5.70	136	*221
		6.87	129	395
		5.71	135	367
Mean		5.98	136	368
SD		0.592	6.16	26.0
%CV		9.9	4.5	7.1
%Bias		-6.7	3.0	6.7
n		4	4	3

* Sample deactivated due to a sample preparation error.

** > ±20% deviation from theoretical

Table 139. M556 Quality Controls (Serum vs. Plasma)

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC (Plasma) 4.00 ppb	Medium QC (Plasma) 150 ppb	High QC (Plasma) 400 ppb
27-Oct-2000	PFOSMD-40	3.73	133	389
		3.45	168	420
		3.34	139	387
Mean		3.51	147	399
SD		0.201	18.7	18.5
%CV		5.7	12.7	4.6
%Bias		-12.3	-2.0	-0.3
n		3	3	3
Run Date	**Run Number**	**Low QC (Serum) 5.30 ppb**	**Medium QC (Serum) 151 ppb**	**High QC (Serum) 401 ppb**
27-Oct-2000	PFOSMD-46	5.03	166	383
		5.23	152	**243
		**4.15	152	371
		5.01	155	388
Mean		4.86	156	381
SD		0.480	6.65	8.74
%CV		9.9	4.3	2.3
%Bias		-8.3	3.3	-5.0
n		4	4	3

** > ±20% deviation from theoretical

Table 140. M570 Quality Controls (Serum vs. Plasma)

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC (Plasma) 4.00 ppb	Medium QC (Plasma) 150 ppb	High QC (Plasma) 400 ppb
27-Oct-2000	PFOSMD-40	3.95	133	379
		3.54	161	403
		3.38	136	360
Mean		3.62	143	381
SD		0.294	15.4	21.5
%CV		8.1	10.8	5.6
%Bias		-9.5	-4.7	-4.8
n		3	3	3
Run Date	**Run Number**	**Low QC (Serum) 6.70 ppb**	**Medium QC (Serum) 153 ppb**	**High QC (Serum) 403 ppb**
27-Oct-2000	PFOSMD-46	6.36	169	397
		6.35	150	*227
		**5.33	156	367
		6.39	155	400
Mean		6.11	158	388
SD		0.519	8.10	18.2
%CV		8.5	5.1	4.7
%Bias		-8.8	3.3	-3.7
n		4	4	3

* Sample deactivated due to a sample preparation error.

** > ±20% deviation from theoretical

Table 140. M570 Quality Controls (Serum vs. Plasma) Continued

All concentrations are expressed as ppb.

Run Date	Run Number	Low QC (Plasma) 4.00 ppb	Medium QC (Plasma) 150 ppb	High QC (Plasma) 400 ppb
25-Apr-2001	25	3.24	180	420
		3.49	175	417
		3.58	164	440
		4.05	171	432
Mean		3.59	173	427
SD		0.339	6.76	10.7
%CV		9.4	3.9	2.5
%Bias		-10.3	15.3	6.8
n		4	4	4
Run Date	**Run Number**	**Low QC (Serum) 6.70 ppb**	**Medium QC (Serum) 153 ppb**	**High QC (Serum) 403 ppb**
25-Apr-2001	26	5.90	166	392
		7.49	167	445
		7.03	167	437
		8.03	161	409
Mean		7.11	165	421
SD		0.906	2.87	24.6
%CV		12.7	1.7	5.8
%Bias		6.1	7.8	4.5
n		4	4	4

** > ±20% deviation from theoretical

Table 141. Serum QC Results Based on Plasma Curves

Sample ID	PFOS (ppb)	PFOSA (ppb)*	PFOSAA (ppb)*	POAA (ppb)	PFHS (ppb)*	M556 (ppb)*	M570 (ppb)
Low (analyzed 10/27/00)	41.2			11.1	7.64	4.44	6.99
Low (analyzed 10/27/00)	41.8			10.8	7.71	4.61	6.98
Low (analyzed 10/27/00)	41.8			10.1	9.22	3.67	5.93
Low (analyzed 10/27/00)	42.1			11.4	7.72	4.42	7.02
Low (analyzed 4/25/01)	41.7	5.04	6.68	9.62			6.41
Low (analyzed 4/25/01)	46.5	6.47	8.5	9.93			7.74
Low (analyzed 4/25/01)	46.7	6.75	8.72	11.3			8.29
Low (analyzed 4/25/01)	47.0	6.88	8.83	13.0			8.94
mean *	43.6	6.29	8.18	10.9	8.07	4.29	7.29
std. dev. *	2.61	0.847	1.01	1.07	0.766	0.419	0.986
%CV *	6.0%	13.5%	12.4%	9.9%	9.5%	9.8%	13.5%
%dev *	10.9%	57.1%	13.6%	14.3%	25.9%	-19.2%	8.8%
acceptance range *	31.4 to 47.2	3.00 to 5.00	5.40 to 9.00	7.63 to 11.4	5.13 to 7.69	4.24 to 6.36	5.36 to 8.04
95% confidence range *	**41.8 to 45.4**	**5.45 to 7.12**	**7.48 to 8.88**	**10.2 to 11.7**	**7.32 to 8.82**	**3.87 to 4.7**	**6.60 to 7.97**
Med (analyzed 10/27/00)	182			186	180	149	175
Med (analyzed 10/27/00)	168			172	170	136	155
Med (analyzed 10/27/00)	165			174	162	137	161
Med (analyzed 10/27/00)	168			177	169	139	160
Med (analyzed 4/25/01)	185	205	192	160			184
Med (analyzed 4/25/01)	189	207	200	164			190
Med (analyzed 4/25/01)	191	210	203	164			191
Med (analyzed 4/25/01)	199	220	207	170			191
mean *	181	211	201	171	170	140	176
std. dev. *	12.5	6.66	6.4	8.37	7.41	5.97	15.3
%CV *	6.9%	3.2%	3.2%	4.9%	4.4%	4.3%	8.7%
%dev *	12.3%	40.3%	31.0%	19.5%	29.0%	-7.1%	15.0%
acceptance range *	129 to 193	113 to 188	115 to 191	114 to 172	106 to 158	121 to 181	122 to 184
95% confidence range *	**172 to 190**	**204 to 217**	**196 to 205**	**165 to 177**	**163 to 178**	**134 to 146**	**165 to 186**
High (analyzed 10/27/00)	364			441	400	361	416
High (analyzed 10/27/00)	370			443	452	349	384
High (analyzed 10/27/00)	374			459	424	366	420
High (analyzed 4/25/01)	396	493	451	453			433
High (analyzed 4/25/01)	399	509	467	470			450
High (analyzed 4/25/01)	406	543	490	476			478
High (analyzed 4/25/01)	424	560	506	478			486
mean *	390	526	479	460	425	359	438
std. dev. *	21.8	30.7	24.3	15.2	26.0	8.74	36.0
%CV *	5.6%	5.8%	5.1%	3.3%	6.1%	2.4%	8.2%
%dev *	5.5%	31.6%	18.7%	24.3%	23.3%	-10.6%	8.7%
acceptance range *	296 to 444	300 to 500	302 to 504	296 to 444	276 to 414	321 to 481	322 to 484
95% confidence range *	**374 to 407**	**492 to 561**	**460 to 497**	**449 to 471**	**396 to 455**	**349 to 369**	**411 to 465**

* Due to rounding, calculations based on displayed values may differ from actual.

NOTE: Only data from acceptable runs included in the Table (see report text).

(Data obtained on 10/27/00 from run PFOSMD-40. Data obtained on 4/25/01 from run S00040-25.)