ANCILLARY STUDIES - EXPOSOMIC ASSAYS

1. Non-targeted Exposomic Assay

Description: This assay is designed to broadly capture environmental exposures and transformations in biological samples. The unbiased liquid chromatography-high resolution tandem mass spectrometry (LC-HRMS/MS) discovery workflow generates molecular fingerprints reflecting health, disease, or exposure states without requiring prior knowledge of the compounds involved. Results are qualitative and allow relative comparisons across samples rather than absolute quantification. Annotations are assigned with confidence levels using an evidence-based classification system based on community guidelines.

Exposure Compound Database: This assay uses a curated library of ~500 exogenous small molecules including environmental phenols, flame retardants, plastics and plasticizers (and their metabolites), personal care products (phthalates), pesticides and related molecules, and PFAS/PFOS.

Included: The analysis includes sample preparation for exposure compounds, comprehensive quality control assessments using extraction blanks, pooled quality control samples, and isotopically labeled standards, and LC-HRMS/MS acquisition. Data are processed through a pipeline that includes raw data file conversion, retention time alignment, peak detection, normalization, and outlier detection, followed by annotation against the exposure compound database (see explanation of metabolite identification confidence levels).

Deliverables: Reports include detailed sample analysis methods, quality control assessment to demonstrate sample preparation and instrument variability (includes principal component analysis figure to visualize sample clustering across the dataset), and a candidate exposure compound annotation data table that includes normalized relative abundance values for each compound annotated in individual samples.

Cost: $245 per sample (Vanderbilt University's approved external, not-for-profit rate) with a minimum of 80 samples, or $225 per sample (Vanderbilt University's approved internal rate) with a minimum of 80 samples. Please contact us for pricing on smaller quantities.

2. Targeted Per- and Polyfluoroalkyl Substances (PFAS) Quantitative Assay

Description: This assay specifically measures per- and polyfluoroalkyl substances (PFAS) using a semi-targeted liquid chromatography high resolution tandem mass spectrometry analysis (LC-HRMS/MS) approach workflow to quantify 57 prioritized species. The method uses data dependent data acquisition coupled with targeted data acquisition, followed by targeted data analysis. The assay uses 19 isotopically labeled PFAS standards to quantify 57 unique PFAS compounds. Accurate quantification using surrogate markers is informed by the FDA guidelines using the M10 bioanalytical method validation and study sample analysis guidance documentation. Measurements

for this panel are adapted from the EPA Method 1633 and previously published studies (e.g., PMID: 29155920, PMID: 36920051, PMID: 35870502) and the method is benchmarked using commercially available NIST standard reference material (SRM 1950: Frozen Human Plasma Standard Reference Material ® 1950 or equivalent). A list of the PFAS species in the quantification assay can be found in **Appendix 1**.

NOTE: The data generated during the PFAS assay acquisition can later be re-analyzed using non-targeted data analysis approaches to search for endogenous metabolites or uncharacterized exposures associated with a given child's health outcome. Blinded duplicate analyses (between 2-10%) should be performed in these studies.

Included: The assay includes PFAS-specific sample preparation, quality control and assessment using blanks, pooled controls, isotopically labeled PFAS standards, and LC-HRMS/MS acquisition combining targeted and semi-targeted data collection. Method validation includes the use of quality control blanks, quality control standard reference materials, and quality control calibration curves.

Deliverables: Reports include detailed sample analysis methods, a detailed quantification table and performance metrics to demonstrate accuracy and reproducibility of the method. The output includes percent coefficient of variation for quality control pooled samples (to assess precision), percent mean recovery of NIST standard reference material 1950 (or equivalent, to assess accuracy), calculated limits of detection (LOD) and quantification (by regression or matrix matched spiked samples), quantification values (ng/mL) for each PFAS compound in individual samples, percent detection frequency of PFAS species detected across the sample set, and, if blinded duplicates are available, relative percent difference will be calculated to assess reproducibility.

Cost: $275 per sample (Vanderbilt University's approved external, not-for-profit rate) with a minimum of 80 samples, or $250 per sample (Vanderbilt University's approved internal rate) with a minimum of 80 samples. Please contact us for pricing on smaller quantities.

3. Non-targeted Nutrition Assay

Description: This non-targeted assay profiles endogenous and exogenous small molecules providing a systems-level view of biological processes. An unbiased liquid chromatography high resolution tandem mass spectrometry (LC-HRMS/MS) approach captures a broad spectrum of metabolites in biological samples. This discovery-based analysis detects unexpected or unknown metabolite changes and allows one to build broad metabolome profiles without prior assumptions. The results are qualitative, allowing relative comparisons of compound abundance across samples. Compound annotations are assigned with confidence levels using an evidence-based classification system based on community guidelines.

Nutrition Compound Database: The metabolomic workflow is supported by a composite database that incorporates MassBank, mzCloud, HMDB, KEGG, GNPS, LipidMaps, MS-Dial, and includes a highly curated in-house reference set (~1000

molecules). The library includes both endogenous metabolites from primary and secondary pathways and exogenous nutrition and food compounds.

Included: The analysis includes metabolite-specific sample preparation, quality control with blanks, pooled controls, and isotopically labeled standards, and LC-HRMS/MS acquisition. Data are processed through a pipeline that includes raw data file conversion, retention time alignment, peak detection, normalization, and outlier detection, followed by annotation against the metabolite database (see explanation of metabolite identification confidence levels).

Deliverables: Reports include detailed sample analysis methods, quality control assessment to demonstrate sample preparation and instrument variability (includes a principal component analysis figure to visualize sample clustering across the dataset), and a candidate metabolite annotation (both exogenous and endogenous compounds) data table that includes normalized relative abundance values for each compound annotated in individual samples.

Cost: $245 per sample (Vanderbilt University's approved external, not-for-profit rate) with a minimum of 80 samples, or $225 per sample (Vanderbilt University's approved internal rate) with a minimum of 80 samples. Please contact us for pricing on smaller quantities.

4. Non-targeted Metabolomic Assay

Description: This non-targeted assay profiles endogenous and exogenous small molecules providing a systems-level view of biological processes. An unbiased liquid chromatography high resolution tandem mass spectrometry (LC-HRMS/MS) approach captures a broad spectrum of metabolites in biological samples. This discovery-based analysis detects unexpected or unknown metabolite changes and allows one to build broad metabolome profiles without prior assumptions. The results are qualitative, allowing relative comparisons of compound abundance across samples. Compound annotations are assigned with confidence levels using an evidence-based classification system based on community guidelines.

Metabolite Compound Database: The metabolomic workflow is supported by a composite database that incorporates MassBank, mzCloud, HMDB, KEGG, GNPS, LipidMaps, MS-Dial, and includes a highly curated in-house reference set (~1000 molecules). The library includes both endogenous metabolites from primary and secondary pathways and exogenous molecules from diet, drugs, and microbial species.

Included: The analysis includes metabolite-specific sample preparation, quality control with blanks, pooled controls, and isotopically labeled standards, and LC-HRMS/MS acquisition. Data are processed through a pipeline that includes raw data file conversion, retention time alignment, peak detection, normalization, and outlier detection, followed by annotation against the metabolite database (see explanation of metabolite identification confidence levels).

Deliverables: Reports include detailed sample analysis methods, quality control assessment to demonstrate sample preparation and instrument variability (includes a principal component analysis figure to visualize sample clustering across the dataset),

and a candidate metabolite annotation (both exogenous and endogenous compounds) data table that includes normalized relative abundance values for each compound annotated in individual samples.

Cost: $245 per sample (Vanderbilt University's approved external, not-for-profit rate) with a minimum of 80 samples, or $225 per sample (Vanderbilt University's approved internal rate) with a minimum of 80 samples. Please contact us for pricing on smaller quantities.

5. Non-targeted Lipidomic Assay

Description: This assay characterizes lipid species with a discovery-based comprehensive liquid chromatography high resolution tandem mass spectrometry analysis (LC-HRMS/MS) workflow that profiles a broad range of lipids from a biological sample. As an unbiased approach, the assay is aimed at detecting unexpected or unknown lipid changes supporting the development of a profile representative of health, disease, exposure, or outcomes. The results are qualitative, allowing relative comparisons of compound abundance across samples. Compound annotations are assigned with confidence levels using an evidence-based classification system based on community guidelines.

Lipid Compound Database: The lipid database integrates MS-Dial, Lipid Annotator, LipidMatch, and an in-house reference lipid dataset (lipid atlas). It covers major lipid classes including glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, and fatty acyls.

Included: The analysis includes lipid-specific sample preparation using liquid-liquid extraction, quality control with blanks, pooled controls, isotopically labeled standards, and LC-HRMS/MS acquisition. Data are processed through a pipeline that includes retention time alignment, peak detection, normalization, and outlier detection, followed by annotation against the curated lipid databases (see explanation of metabolite identification confidence levels).

Deliverables: Reports include detailed sample analysis methods, quality control assessment to demonstrate sample preparation and instrument variability (includes principal component analysis figure to visualize sample clustering across the dataset, and a candidate lipid annotation data table that includes normalized relative abundance values for each compound annotated in individual samples.

Cost: $245 per sample (Vanderbilt University's approved external, not-for-profit rate) with a minimum of 80 samples, or $225 per sample (Vanderbilt University's approved internal rate) with a minimum of 80 samples. Please contact us for pricing on smaller quantities.

Appendix 1: Current PFAS assay panel and associated isotopically labeled standards used for accurate quantification.

	Compound name	Compound name acronym	Internal Standard Used for Accurate Quantification	Internal Standard Used for Accurate Quantification (Acronym)
1	br-N-ethyl-perfluoro-1-octanesulfonamidoacetic acid	br-NEtFOSAA	Perfluoro-1-(1,2,3,4-$^{13}C_4$)octanesulfonate	PFOS-13C4
2	br-N-methyl-perfluoro-1-octanesulfonamidoacetic acid	br-NMeFOSAA	Perfluoro-1-(1,2,3,4-$^{13}C_4$)octanesulfonate	PFOS-13C4
3	Perfluorooctanesulfonamide	FOSA-I	Perfluoro-1-(1,2,3,4-$^{13}C_4$)octanesulfonate	PFOS-13C4
4	N-ethylperfluoro-1-octanesulfonamide	N-EtFOSA-M	Perfluoro-1-(1,2,3,4-$^{13}C_4$)octanesulfonate	PFOS-13C4
5	N-ethyl-perfluoro-1-octanesulfonamidoacetic acid	N-EtFOSAA	Perfluoro-1-(1,2,3,4-$^{13}C_4$)octanesulfonate	PFOS-13C4
6	2-(N-ethylperfluoro-1-octanesulfonamido)-ethanol	N-EtFOSE-M	Perfluoro-1-(1,2,3,4-$^{13}C_4$)octanesulfonate	PFOS-13C4
7	N-methylperfluoro-1-octanesulfonamide	N-MeFOSA-M	Perfluoro-1-(1,2,3,4-$^{13}C_4$)octanesulfonate	PFOS-13C4
8	2-(N-methylperfluoro-1-octanesulfonamido)-ethanol	N-MeFOSE-M	Perfluoro-1-(1,2,3,4-$^{13}C_4$)octanesulfonate	PFOS-13C4
9	N-methyl-perfluoro-1-octanesulfonamidoacetic acid	N-MeFOSAA	Perfluoro-1-(1,2,3,4-$^{13}C_4$)octanesulfonate	PFOS-13C4
10	2-Perfluorodecyl ethanoic acid (10:2)	FDEA	Perfluoro-n-(1,2-$^{13}C_2$)dodecanoic acid	PFDoA-13C2
11	2H-perfluoro-2-dodecenoic acid	FDUEA	Perfluoro-n-(1,2-$^{13}C_2$)dodecanoic acid	PFDoA-13C2
12	2-Perfluorohexyl ethanoic acid (6:2)	FHEA	Perfluoro-n-(1,2-$^{13}C_2$)hexanoic acid	PFHxA-13C2
13	3-Perfluoroheptyl propanoic acid (7:3)	FHpPA	Perfluoro-n-(1,2-$^{13}C_2$)hexanoic acid	PFHxA-13C2
14	2H-perfluoro-2-octenoic acid	FHUEA	Perfluoro-n-(1,2,3,4-$^{13}C_4$)octanoic acid	PFOA-13C4
15	2-Perfluorooctyl ethanoic acid (8:2)	FOEA	Perfluoro-n-(1,2,3,4-$^{13}C_4$)octanoic acid	PFOA-13C4
16	2H-perfluoro-2-decenoic acid	FOUEA	Perfluoro-n-(1,2,3,4-$^{13}C_4$)octanoic acid	PFOA-13C4
17	3-Perfluoropentyl propanoic acid (5:3)	FPePA	Perfluoro-n-(1,2-$^{13}C_2$)hexanoic acid	PFHxA-13C2
18	3-Perfluoropropyl propanoic acid	FPrPA	Perfluoro-n-(1,2-$^{13}C_2$)hexanoic acid	PFHxA-13C2
19	Fluorotelomer 10:2 sulfonic acid	10:2FTS	Perfluoro-1-hexane($^{18}O_2$)sulfonate	PFOS-13C4
20	Fluorotelomer 4:2 sulfonic acid	4:2FTS	Perfluoro-1-hexane($^{18}O_2$)sulfonate	PFHxS-18O2
21	Fluorotelomer 6:2 sulfonic acid	6:2FTS	Perfluoro-1-hexane($^{18}O_2$)sulfonate	PFHxS-18O2
22	Fluorotelomer 8:2 sulfonic acid	8:2FTS	Perfluoro-1-hexane($^{18}O_2$)sulfonate	PFOS-13C4
23	br-Perfluoro-n-octanoic acid	br-PFOA	Perfluoro-n-(1,2,3,4-$^{13}C_4$)octanoic acid	PFOA-13C4
24	Perfluoro-n-butanoic acid	PFBA	Perfluoro-n-($^{13}C_4$)butanoic acid	PFBA-13C4
25	Perfluoro-n-decanoic acid	PFDA*	Perfluoro-n-(1,2-$^{13}C_2$)decanoic acid	PFDA-13C2
26	Perfluoro-n-dodecanoic acid	PFDoA	Perfluoro-n-(1,2-$^{13}C_2$)dodecanoic acid	PFDoA-13C2
27	Perfluoro-n-heptanoic acid	PFHpA	Perfluoro-n-(1,2-$^{13}C_2$)hexanoic acid	PFHxA-13C2
28	Perfluoro-n-hexanoic acid	PFHxA	Perfluoro-n-(1,2-$^{13}C_2$)hexanoic acid	PFHxA-13C2
29	Perfluoro-n-hexadecanoic acid	PFHxDA	Perfluoro-n-(1,2-$^{13}C_2$)dodecanoic acid	PFDoA-13C2
30	Perfluoro-n-nonanoic acid	PFNA*	Perfluoro-n-(1,2,3,4,5-$^{13}C_5$)nonanoic acid	PFNA-13C5
31	Perfluoro-n-octanoic acid	PFOA*	Perfluoro-n-(1,2,3,4-$^{13}C_4$)octanoic acid	PFOA-13C4
32	Perfluoro-n-octadecanoic acid	PFODA	Perfluoro-n-(1,2-$^{13}C_2$)dodecanoic acid	PFDoA-13C2
33	Perfluoro-n-pentanoic acid	PFPeA	Perfluoro-n-(1,2-$^{13}C_2$)hexanoic acid	PFHxA-13C2
34	Perfluoropropanoic acid	PFPrA	Perfluoro-n-($^{13}C_4$)butanoic acid	PFBA-13C4
35	Perfluoro-n-tetradecanoic acid	PFTeDA	Perfluoro-n-(1,2-$^{13}C_2$)dodecanoic acid	PFDoA-13C2

36	Perfluoro-n-tridecanoic acid	PFTrDA	Perfluoro-n-(1,2-$^{13}C_2$)dodecanoic acid	PFDoA-13C2
37	Perfluoroundecanoic acid	PFUdA*	Perfluoro-n-(1,2-$^{13}C_2$)undecanoic acid	PFUdA-13C2
38	Potassium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate	11Cl-PF3OUdS	Perfluoro-n-(1,2-$^{13}C_2$)dodecanoic acid	PFDoA-13C2
39	Potassium 9-chlorohexadecafluoro-3-oxanonane-2-sulfonate	9Cl-PF3ONS	Perfluoro-n-(1,2-$^{13}C_2$)dodecanoic acid	PFDoA-13C2
40	5-[2-(Aminocarbonyl)hydrazinylidene]-2,3,5,6-tetrahydro-1-methyl-6-oxo-1H-indole-2-Sulfonic Acid Sodium Salt	NaDONA	Perfluoro-n-(1,2-$^{13}C_2$)hexanoic acid	PFHxA-13C2
41	Azane, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid	HFPO-DA	Perfluoro-n-(1,2-$^{13}C_2$)hexanoic acid	PFHxA-13C2
42	Perfluoro-3,6-dioxaheptanoic acid	3,6-OPFHpA	Perfluoro-n-(1,2-$^{13}C_2$)hexanoic acid	PFHxA-13C2
43	Perfluoro-4-oxapentanoic acid	PF4OPeA	Perfluoro-n-(1,2-$^{13}C_2$)hexanoic acid	PFHxA-13C2
44	Perfluoro-5-oxahexanoic acid	PF5OHxA	Perfluoro-n-(1,2-$^{13}C_2$)hexanoic acid	PFHxA-13C2
45	Potassium perfluoro(2-ethoxyethane)sulfonate	PFEESA	Perfluoro-n-(1,2-$^{13}C_2$)hexanoic acid	PFHxA-13C2
46	br-Perfluoro-1-hexanesulfonic acid	br-PFHxS	Perfluoro-1-hexane($^{18}O_2$)sulfonate	PFHxS-18O2
47	br-Perfluoro-1-octanesulfonic acid	br-PFOS	Perfluoro-1-(1,2,3,4-$^{13}C_4$)octanesulfonate	PFOS-13C4
48	Perfluoro-4-ethylcyclohexane sulfonic acid	PFECHS	Perfluoro-1-hexane($^{18}O_2$)sulfonate	PFHxS-18O2
49	Perfluoro-1-butanesulfonic acid	L-PFBS	Perfluoro-1-hexane($^{18}O_2$)sulfonate	PFHxS-18O2
50	Perfluoro-1-dodecanesulfonic acid	L-PFDoS	Perfluoro-1-(1,2,3,4-$^{13}C_4$)octanesulfonate	PFOS-13C4
51	Perfluoro-1-decanesulfonic acid	L-PFDS	Perfluoro-1-(1,2,3,4-$^{13}C_4$)octanesulfonate	PFOS-13C4
52	Perfluoroheptanesulfonic acid	L-PFHpS	Perfluoro-1-(1,2,3,4-$^{13}C_4$)octanesulfonate	PFOS-13C4
53	Perfluoro-1-hexanesulfonic acid	L-PFHxS*	Perfluoro-1-hexane($^{18}O_2$)sulfonate	PFHxS-18O2
54	Perfluoro-1-nonanesulfonic acid	L-PFNS	Perfluoro-1-(1,2,3,4-$^{13}C_4$)octanesulfonate	PFOS-13C4
55	Perfluoro-1-octanesulfonic acid	L-PFOS*	Perfluoro-1-(1,2,3,4-$^{13}C_4$)octanesulfonate	PFOS-13C4
56	Perfluoropentanesulfonic acid	L-PFPeS	Perfluoro-1-hexane($^{18}O_2$)sulfonate	PFHxS-18O2
57	Perfluoropropanoicsulfonic acid	L-PFPrS	Perfluoro-1-hexane($^{18}O_2$)sulfonate	PFHxS-18O2
		*PFAS molecules measured in SRM 1950		