Attachment 2. Biochemical Analytical Plan in Children and Adults.

Biochemical analytical plan in children and adults: performing laboratories, reference levels, reporting ranges, clinical guidelines, and critical values.

Laboratory and Contact	Analyte	* CLIA Cert.	Matrix	Volume	NHANES [1] (µg/L) 2013 - 2014	
Children and Adults						
NCEH/Division of Laboratory Sciences* *Contact: Dr. Antonia Calafat*	*Per- and Poly-fluoroalkyl Substances (PFAS)*	Yes	Serum	1 ml (for all PFAS); 1 ml reserve (for future PFAS analyses)	**Age Group (years):**	**50th to 95th %**
	perfluorooctanoic acid (PFOA)‡				3-5: 6-11: 12-19: 20+:	1.80 – 5.58 1.94 – 3.84 1.67 – 3.47 2.07 – 5.60
	n-PFOA - linear isomer				3-5: 6-11: 12-19: 20+:	1.72 – 5.32 1.84 – 3.77 1.60 – 3.40 2.00 – 5.40
	Sb-PFOA - serum branched isomer				3-5: 6-11: 12-19: 20+:	< LOD – 0.280 < LOD – 0.230 < LOD – 0.200 < LOD – 0.200
	perfluorooctane sulfonic acid, (PFOS)‡				3-5: 6-11: 12-19: 20+:	3.41 – 8.82 4.02 – 12.4 3.60 – 9.30 5.60 – 19.5
	n-PFOS – linear isomer				3-5: 6-11: 12-19: 20+:	2.11 – 6.19 2.65 – 8.41 2.70 – 7.10 3.70 – 15.1
	Sm-PFOS – serum branched				3-5: 6-11: 12-19: 20+:	1.00 – 3.60 1.41 – 4.25 1.00 – 2.30 1.60 – 5.30

Limit of detection (LOD, see Data Analysis section) for Survey year 13-14 is 0.1. < LOD means less than the limit of detection, which may vary for some chemicals by year and by individual sample.

[1] CDC. 2018. 2013-2014 NHANES 50th to 95th percentiles among children 12-19 years and adults 20+ years old from the Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, March 2018. Accessed April 13, 2018 at (https://www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Volume1_Mar2018.pdf).

‡ See Calculation of PFOS and PFOA as the Sum of Isomers for additional information in March 2018 Updated Tables.

Attachment 2. Biochemical Analytical Plan in Children and Adults.

Laboratory and Contact	Analyte	* CLIA Cert.	Matrix	Volume	NHANES [1] (µg/L) 2013 - 2014
Children and Adults					
NCEH/Division of Laboratory Sciences* *Contact: Dr. Antonia Calafat*	*Per- and Poly-fluoroalkyl Substances (PFAS) (continued)*	Yes	Serum	1 ml (for all PFAS); 1 ml reserve (for future PFAS analyses)	**Age Group (years): 50th to 95th %**
	perfluorohexane sulfonic acid (PFHxS)				3-5: 0.740 – 1.62 6-11: 0.850 – 4.14 12-19: 1.10 – 6.30 20+: 1.40 – 5.50
	perfluorooctane sulfonamide (PFOSA)				3-5: < LOD – 0.110 6-11: < LOD - < LOD 12-19: n/a ‡ 20+: n/a ‡
	2-(N-methyl-perfluorooctane sulfonamido) acetic acid (Me-PFOSAA)				3-5: 0.110 – 1.02 6-11: 0.110 – 0.940 12-19: 0.100 – 0.600 20+: < LOD – 0.600
	2-(N-ethyl-perfluorooctane sulfonamido) acetic acid (Et-PFOSAA)				3-5: < LOD - < LOD 6-11: < LOD - < LOD 12-19: n/a ‡ 20+: n/a ‡
	perfluorobutane sulfonic acid (PFBS)				3-5: < LOD - < LOD 6-11: < LOD – 0.130 12-19: < LOD - < LOD 20+: < LOD - < LOD
	perfluoroheptanoic acid (PFHpA)				3-5: < LOD – 0.310 6-11: < LOD – 0.170 12-19: < LOD – 0.200 20+: < LOD – 0.100

Limit of detection (LOD, see Data Analysis section) for Survey year 13-14 is 0.1. < LOD means less than the limit of detection, which may vary for some chemicals by year and by individual sample. ‡ Not measured after Survey Years 2011-2012.

[1] CDC. 2018. 2013-2014 NHANES 50th to 95th percentiles among children 12-19 years and adults 20+ years old from the Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, March 2018. Accessed April 13, 2018 at (https://www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Volume1_Mar2018.pdf).

Attachment 2. Biochemical Analytical Plan in Children and Adults.

Laboratory and Contact	Analyte	* CLIA Cert.	Matrix	Volume	NHANES [1] (µg/L) 2013 - 2014
Children and Adults					
NCEH/Division of Laboratory Sciences* *Contact: Dr. Antonia Calafat*	*Per- and Poly-fluoroalkyl Substances (PFAS) (continued)*	Yes	Serum	1 ml (for all PFAS); 1 ml reserve (for future PFAS analyses)	**Age Group (years): 50th to 95th %**
	perfluorononanoic acid (PFNA)				3-5: 0.620 – 3.49 6-11: 0.750 – 3.19 12-19: 0.500 – 2.00 20+: 0.700 – 2.00
	perfluorodecanoic acid (PFDA)				3-5: 0.100 – 0.370 6-11: < LOD – 0.350 12-19: 0.100 – 0.400 20+: 0.193 – 0.800
	perfluoroundecanoic acid (PFUnDA)				3-5: < LOD – 0.370 6-11: < LOD – 0.250 12-19: < LOD – 0.200 20+: < LOD – 0.500
	perfluorododecanoic acid (PFDoA)				3-5: < LOD - < LOD 6-11: < LOD - < LOD 12-19: < LOD – 0.200 20+: < LOD – 0.200
Laboratory and Contact	**Proposed Biospecimen Bank for Future Analytes**	*** CLIA Cert.**	**Matrix**	**Volume**	**NHANES [TBD] (µg/L) 20xx – 20xx**
Children and Adults					
NCEH/Division of Laboratory Sciences* *Contact: Dr. Antonia Calafat*	*Per- and Poly-fluoroalkyl Substances (PFAS)*	Yes	Spot Urine (morning void)	1 ml (for PFAS); 15 ml for creatinine/or specific gravity)	**Age Group: 50th to 95th %**
	To be determined (TBD) when analytical methods are developed (Including but not limited to the following 18 analytes: PFOA [n-PFOA;, Sb-PFOA], PFOA [n-PFOS, Sm-PFOS], PFHxS, PFBS, PFHpA, PFNA, PFDA, PFUnDA, PFPrS, PFHpS, PFBA, PFPeA, PFHxA, HFPO-DA (GenX), DONA, 9Cl-PF3ONS)				3-5: TBD 6-11: TBD 12-19: TBD 20+: TBD
	Creatinine (for urinary creatinine correction; may be contracted)				TBD

Limit of detection (LOD, see Data Analysis section) for Survey year 13-14 is 0.1. < LOD means less than the limit of detection, which may vary for some chemicals by year and by individual sample. ‡ Not measured after Survey Years 2011-2012.

[1] CDC. 2018. 2013-2014 NHANES 50th to 95th percentiles among children 12-19 years and adults 20+ years old from the Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, March 2018. Accessed April 13, 2018 at (https://www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Volume1_Mar2018.pdf).

Attachment 2. Biochemical Analytical Plan in Children and Adults.

Laboratory and Contact	Analyte	* CLIA Cert.	Matrix	Volume	Reportable Range, Guidelines, Critical Values *Reference ranges will be updated when commercial lab is selected.*
Children and Adults					
Commercial Laboratory (to be determined)* *Contact:*	*Lipids*	Yes	Serum	0.5 ml (for all)	
	Total cholesterol, fasting				Coronary Heart Disease Risk (CHD)[1] Adult, 18+ years: Desirable: <200 mg/dL Borderline High: 200-239 mg/dL High: ≥240 mg/dL Child, 2-17 years: Acceptable: <170 mg/dL Borderline high: 170-199 mg/dL High: ≥200 mg/dL
	Triglycerides, fasting				CHD Risk[1] Adult, 18+ years: Normal: <150 mg/dL Borderline High: 150-199 mg/dL High: 200-499 mg/dL Very High: ≥500 mg/dL **Critical Value: >1,000 mg/dL** Child, 2-9 years: Acceptable: <75 mg/dL Borderline high: 75-99 mg/dL High: ≥100 mg/dL Child, 10-17 years: Acceptable: <90 mg/dL Borderline high: 90-129 mg/dL High: > or =130 mg/dL
	Low Density Lipoprotein (LDL), fasting				CHD Risk[1] Adult, 18+ years:

[1] https://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/8320

Attachment 2. Biochemical Analytical Plan in Children and Adults.

					Desirable: <100 mg/dL Above Desirable: 100-129 mg/dL Borderline high: 130-159 mg/dL High: 160-189 mg/dL Very high: ≥190 mg/dL Child, 2-17 years: Acceptable: <110 mg/dL Borderline high: 110-129 mg/dL High: ≥130 mg/dL
	High Density Lipoprotein (HDL), fasting				<u>CHD Risk</u>[1] Adult, 18+ years: Males: ≥40 mg/dL Females: ≥50 mg/dL Child, 2-17 years: Low: <40 mg/dL Borderline low: 40-45 mg/dL Acceptable: > 45 mg/dL

Attachment 2. Biochemical Analytical Plan in Children and Adults.

Laboratory and Contact	Analyte	* CLIA Cert.	Matrix	Volume	Reportable Range, Guidelines, Critical Values *Reference ranges will be updated when commercial lab is selected.*
Children and Adults					
Commercial Laboratory (to be determined)* *Contact:*	*Uric Acid*	Yes	Serum	1 ml	Males[2] ≤ 8.0 mg/dL Females ≤ 6.1 mg/dL
	Creatinine (to estimate glomerular filtration rate [eGFR])				Males[3] 1-2 years: 0.1-0.4 mg/dL 3-4 years: 0.1-0.5 mg/dL 5-9 years: 0.2-0.6 mg/dL 10-11 years: 0.3-0.7 mg/dL 12-13 years: 0.4-0.8 mg/dL 14-15 years: 0.5-0.9 mg/dL > or =16 years: 0.8-1.3 mg/dL Reference values have not been established for patients that are <12 months of age. Females 1-3 years: 0.1-0.4 mg/dL 4-5 years: 0.2-0.5 mg/dL 6-8 years: 0.3-0.6 mg/dL 9-15 years: 0.4-0.7 mg/dL > or =16 years: 0.6-1.1 mg/dL Reference values have not been established for patients that are <12 months of age. ESTIMATED GFR >60 mL/min/BSA **Note:** eGFR results will not be calculated for patients <18 or >70 years old.

[2] https://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/8440
[3] https://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/8472

Attachment 2. Biochemical Analytical Plan in Children and Adults.

Laboratory and Contact	Analyte	* CLIA Cert.	Matrix	Volume	Reportable Range, Guidelines, Critical Values *Reference ranges will be updated when commercial lab is selected.*
Children and Adults					
Commercial Laboratory (to be determined)* *Contact:*	*Thyroid Hormones*	Yes	Serum	0.5 ml	
	Thyroid Stimulating Hormone (TSH)				0.30-3.0 mIU/L [4]
	Free Total Thyroxine (Free T4)				0.8-2.0 ng/dL
	Total Thyroxine (TT4)				4.5-12.5 µg/dL
	Total Triiodothyronine (TT3)				80-180 ng/dL
Commercial Laboratory (to be determined)* *Contact:*	*Liver Tests*	Yes	Serum	0.5 ml standard tests; 1 ml CK18	
	Alanine transaminase (ALT)				15-65 U/L [5]
	Aspartate transaminase (AST)				5-40 U/L
	Alkaline phosphatase (ALP)				Female: 50-136 U/L; Male: 40-136 U/L
	Gamma-glutamyltransferase (GGT)				Female 5-55 U/L; Male 5-85 U/L
	Albumin (Alb)				3.4-5.0 g/dL **Critical Value: <1.5 g/dL** **Critical Value: >7.9 g/dL**
	Total bilirubin (TBIL)				0.0 – 1.0 mg/dL **Critical Value: >12.9 mg/dL**
	Direct bilirubin (Conjugated Bilirubin)				0.0-0.3 mg/dL
	Non-alcoholic fatty liver disease (NAFLD)/steatohepatitis				
	Cytokeratin 18 M30 (CK-18 M30) Cytokeratin 18 M65 (CK-18 M65)				No evident liver disease: M30 <200 U/L and M65 <300 U/L TASH: M30<200 U/L and M65 >300 U/L Other liver disease: M30: >200 U/L

[4] University of Southern California Clinical Laboratories Endocrine Services.

[5] University of Louisville Department of Medicine, Gastroenterology (updated 14 October 2015).

Attachment 2. Biochemical Analytical Plan in Children and Adults.

Laboratory and Contact	Analyte	* CLIA Cert.	Matrix	Volume	Reportable Range, Guidelines, Critical Values *Reference ranges will be updated when commercial lab is selected.*
Children and Adults					
Commercial Laboratory (to be determined)* *Contact:*	*Sex Hormones*	Yes	Serum	1 ml	
	Testosterone				Males[6] 4-9 years: <7-20 ng/dL 10-11 years: <7-130 ng/dL 12-13 years: <7-800 ng/dL 14 years: <7-1,200 ng/dL 15-16 years: 100-1,200 ng/dL 17-18 years: 300-1,200 ng/dL ≥19 years: 240-950 ng/dL Females 4-9 years: <7-20 ng/dL 10-11 years: <7-44 ng/dL 12-16 years: <7-75 ng/dL 17-18 years: 20-75 ng/dL ≥19 years: 8-60 ng/dL
	Estradiol				CHILDREN[7] Males **Tanner Stages** — **Reference Range** Stage I (>14 days and prepubertal) — <LOD-13 pg/mL Stage II — <LOD-16 pg/mL Stage III — <LOD-26 pg/mL Stage IV — <LOD-38 pg/mL Stage V — 10-40 pg/mL Females **Tanner Stages** — **Mean Age**

[6] https://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/83686
[7] https://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/81816

Attachment 2. Biochemical Analytical Plan in Children and Adults.

					Stage I (>14 days and prepubertal) 7.1 years Stage II 10.5 years Stage III 11.6 years Stage IV 12.3 years Stage V 14.5 years ADULTS Males: 10-40 pg/mL Females Premenopausal: 15-350 pg/mL** Postmenopausal: <10 pg/mL **E2 levels vary widely through the menstrual cycle.
	Sex hormone-binding globulin (SHBG)				CHILDREN[8] Males Tanner Stages / Reference Range Stage I 31-167 nmol/L Stage II 49-179 nmol/L Stage III 5.8-182 nmol/L Stage IV 14-98 nmol/L Stage V 10-57 nmol/L Females Tanner Stages / Reference Range Stage I 43-197 nmol/L Stage II 7.7-119 nmol/L Stage III 31-191 nmol/L Stage IV 31-166 nmol/L Stage V 18-144 nmol/L ADULTS Males: 10-57 nmol/L Females (non-pregnant): 18-144 nmol/L

[8] https://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/9285

Attachment 2. Biochemical Analytical Plan in Children and Adults.

	Follicle stimulating hormone (FSH)				Males[9] 4-6 years: < or =6.7 IU/L 7-8 years: < or =4.1 IU/L 9-10 years: < or =4.5 IU/L 11 years: 0.4-8.9 IU/L 12 years: 0.5-10.5 IU/L 13 years: 0.7-10.8 IU/L 14 years: 0.5-10.5 IU/L 15 years: 0.4-18.5 IU/L 16 years: < or =9.7 IU/L 17 years: 2.2-12.3 IU/L ≥18 years: 1.0-18.0 IU/L Females 15 days-6 years: < or =3.3 IU/L 7-8 years: < or =11.1 IU/L 9-10 years: 0.4-6.9 IU/L 11 years: 0.4-9.0 IU/L 12 years: 1.0-17.2 IU/L 13 years: 1.8-9.9 IU/L 14-16 years: 0.9-12.4 IU/L 17 years: 1.2-9.6 IU/L ≥18 years: Premenopausal Follicular: 3.9-8.8 IU/L Midcycle: 4.5-22.5 IU/L Luteal: 1.8-5.1 IU/L Postmenopausal: 16.7-113.6 IU/L
	Insulin-like growth factor (IGF-1)				

Laboratory and Contact	**Analyte**	*** CLIA Cert.**	**Matrix**	**Volume**	**Reportable Range, Guidelines, Critical Values** *Reference ranges will be updated when commercial lab is selected.*
Children and Adults					
Commercial	*Immune Function*	Yes	Serum	2 ml	

[9] https://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/8670

Attachment 2. Biochemical Analytical Plan in Children and Adults.

Laboratory (to be determined)* *Contact:*	Ig A, Ig G, Ig M, Ig E				
Commercial Laboratory (to be determined)* *Contact:*	*Glycemic Parameters*	Yes			
	Glycosylated hemoglobin (HbA1c)		Whole Blood EDTA	1 ml; plus 1 ml reserve	Diabetes Risk[10] Normal: <5.7% Increased Risk Diabetes: 5.7-6.4% Diabetes: ≥6.5% (confirmation required)
	Glucose, fasting, 8-hour		Serum	1 ml Glucose/ Insulin; 1 ml antibodies	
	Insulin				<17 µU/ml [8]
	Pro-insulin				3.6-22 pmol/L[8]
	C-peptide				1.1-4.4 ng/mL[8]
	Glutamate Decarboxylase -65 (Anti-GAD 65)				Negative Antibody: DK≤33 [8] Positive Antibody: DK>33
	Thyrosine Phosphatase-like Protein Autoantibodies (Anti-IA2)				Negative Antibody: DK<5 [8] Positive Antibody: DK≥5
Children Only					
Commercial Laboratory (to be determined)* *Contact:*	*Antibodies to measles, mumps, rubella, tetanus, and diphtheria*	Yes	Serum	1 ml	
			Child Total	**Serum - 11ml Whole Blood – 2 ml Urine – 16 ml Red Top 3 x10 ml EDTA Lavender Top 3 ml**	

Adults Only					
Commercial Laboratory (to be determined)* *Contact:*	*Autoimmune Parameters*	Yes	Serum	2 ml (for all)	
	Rheumatoid Factor (RF)				< 15 IU/mL[11]
	Antinuclear Antibody (ANA) screen				< or =1.0 U (negative)[12] 1.1-2.9 U (weakly positive) 3.0-5.9 U (positive) > or =6.0 U (strongly positive)
	Antinuclear Antibody (ANA) titer				

[10] American Diabetes Association. Standards of Medical Care in Diabetes - 2011. Diabetes Care. January 2011;34(Supplement 1):S11-S61 (subject to periodic update).

[11] https://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/9060

[12] https://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/9026

Attachment 2. Biochemical Analytical Plan in Children and Adults.

	Inflammatory Cytokines				
	Interleukin 1-β (IL-1β), IL-4, IL-6, IL-8, IL-12, monocyte chemotactic protein-1 (MCP-1), tumor necrosis factor α (TNFα), leptin, adiponectin, resistin, plasminogen activator inhibitor-1 (PAI-1).	No	Serum	2 ml	Clinical reference levels not established.
			Adult Total	**Serum – 15 ml Whole Blood – 2 ml Urine – 16 ml Red Top 4 x 10 ml EDTA Lavender Top 3 ml**	