Attachment 3. 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	2 ml (for all PFAS)	**Age Group (years): 50th to 95th %**
	perfluorooctanoic acid (PFOA)‡				3-5: 1.80 – 5.58 6-11: 1.94 – 3.84 12-19: 1.67 – 3.47 20+: 2.07 – 5.60
	n-PFOA - linear isomer				3-5: 1.72 – 5.32 6-11: 1.84 – 3.77 12-19: 1.60 – 3.40 20+: 2.00 – 5.40
	Sb-PFOA - serum branched isomer				3-5: < LOD – 0.280 6-11: < LOD – 0.230 12-19: < LOD – 0.200 20+: < LOD – 0.200
	perfluorooctane sulfonic acid, (PFOS)‡				3-5: 3.41 – 8.82 6-11: 4.02 – 12.4 12-19: 3.60 – 9.30 20+: 5.60 – 19.5
	n-PFOS – linear isomer				3-5: 2.11 – 6.19 6-11: 2.65 – 8.41 12-19: 2.70 – 7.10 20+: 3.70 – 15.1
	Sm-PFOS – serum branched				3-5: 1.00 – 3.60 6-11: 1.41 – 4.25 12-19: 1.00 – 2.30 20+: 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 3. 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	2 ml (for all PFAS)	**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
	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

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 3. 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	2 ml (for all PFAS)	**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

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)	7 ml (for all)	**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. ‡‡ Reference ranges for NHANES 2017-2018 are listed at https://www.cdc.gov/exposurereport/pfas_early_release.html;

[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 3. Biochemical Analytical Plan in Children and Adults.

Laboratory and Contact	Analyte	* CLIA Cert.	Matrix	Volume	Reportable Range, Guidelines, Critical Values
Children and Adults					
Commercial Laboratory (to be determined)* *Contact:*	*Lipids*	Yes	Serum	1 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: Desirable: <100 mg/dL

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

Attachment 3. Biochemical Analytical Plan in Children and Adults.

					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 3. Biochemical Analytical Plan in Children and Adults.

Laboratory and Contact	Analyte	* CLIA Cert.	Matrix	Volume	Reportable Range, Guidelines, Critical Values
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.
Laboratory and	**Analyte**	*** CLIA**	**Matrix**	**Volume**	**Reportable Range, Guidelines,**

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

Attachment 3. Biochemical Analytical Plan in Children and Adults.

Contact		Cert.			Critical Values
Children and Adults					
Commercial Laboratory (to be determined)* *Contact:*	*Thyroid Hormones*	Yes	Serum	1 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	2 ml (for all)	
	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
Laboratory and Contact	**Analyte**	*** CLIA Cert.**	**Matrix**	**Volume**	**Reportable Range, Guidelines, Critical Values**

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

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

Attachment 3. Biochemical Analytical Plan in Children and Adults.

<table>
<tr><th colspan="6">Children and Adults</th></tr>
<tr><td rowspan="2">Commercial Laboratory (to be determined)*
Contact:</td><td>Sex Hormones

Testosterone</td><td rowspan="2">Yes</td><td rowspan="2">Serum</td><td rowspan="2">1 ml</td><td>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</td></tr>
<tr><td>Estradiol</td><td>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
Stage I (>14 days and prepubertal) — 7.1 years
Stage II — 10.5 years</td></tr>
</table>

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

Attachment 3. Biochemical Analytical Plan in Children and Adults.

					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
	Follicle stimulating hormone (FSH)				Males[9] 4-6 years: < or =6.7 IU/L

[8] https://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/9285
[9] https://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/8670

Attachment 3. Biochemical Analytical Plan in Children and Adults.

					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**
Children and Adults					
Commercial Laboratory (to be determined)* *Contact:*	*Immune Function*	Yes	Serum	1 ml	
	Ig A, Ig G, Ig M, Ig E				
Commercial	*Glycemic Parameters*	Yes			

Attachment 3. Biochemical Analytical Plan in Children and Adults.

Laboratory (to be determined)* *Contact:*	Glycosylated hemoglobin (HbA1c)		Whole Blood EDTA	2 ml	Diabetes Risk[10] Normal: <5.7% Increased Risk Diabetes: 5.7-6.4% Diabetes: ≥6.5% (confirmation required)
	Glucose, fasting, 8-hour		Serum	2 ml (for all four)	
	Insulin				<17 µU/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 – 7 ml Red Top 20 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				
			Adult Total	**Serum - 13ml Whole Blood – 2 ml Urine – 7 ml Red Top 30 ml EDTA Lavender Top 3 ml**	

[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