

NCS PROTOCOL BIOSPECIMEN DETAILS
June 20, 2007 – Version 1.3

Summary of Biological Specimens by Person/Visit (Mother (M), Father (F), Child (C))

Prepregnancy					Pregnancy							Birth				Postnatal								
P1 Home	Materials left behind and picked up after P1 ¹	P 1 month (Phone)	P 2 months (Phone)	P 4 months (Phone)	T1-1st (Home)	T1-Prior (Home)	Materials left behind and picked up after T1 ¹	16-17 weeks (Phone)	T2 (Clinic)	T3 (Clinic)	Materials left behind and picked up after T3 ¹	36 weeks (Phone)	B1 Delivery (Hospital)	B2 Predischarge (Hospital)	Materials left behind and picked up after B2 ¹	1 month visit if needed (Home)	3 months (Phone)	6 month visit (Home)	Materials left behind and picked up after 6 mo. visit ¹	9 months (Phone)	12 month visit (Home)	Materials left behind and picked up after 12 mo. visit ¹	18 months (Phone)	24 months (Phone)
Blood (M) Vaginal (M) Hair (M) Urine Kit (M) ²	Urine ³	Pregnancy tests plus urine kit (M)			Blood (M,F) Urine kit (M,F) ² Vaginal (M) Hair (M,F) Nail (F) Saliva kit (M) ⁴	Blood (M,F) Urine kit (M,F) ² Vaginal (M) Hair (M,F) Nail (F) Saliva kit (M) ⁴	Urine (M,F) Saliva (M)				Blood (M) Urine ⁵ Vaginal (M) Hair (M) Nail (M) Saliva ⁵		Blood (M) Urine (M) Placenta (M) Umbilical cord (M) Heel stick (C) Cord blood(C)	Meconium (C)		Breast milk kit (M) ⁶	Breast milk kit (M) ⁶	Breast milk (M) ⁶ Urine (C) ² Hair (C) Saliva kit (M,F) ⁴	Saliva (M,F)		Blood (C) Urine (C) ² Hair (C) Saliva kit (C) ⁴	Saliva (C)		

¹ Activity is initiated at in-person visit and requires participant action after the visit (e.g., mail-in, self-collected urine sample, complete and mail self-administered questionnaire). Time frame for completion varies and is specific to each activity.

² Urine will be self collected as a first morning void (except at birth). At participant visits, a kit will be left and the participant will collect a first morning void urine the morning of the sample pickup.

³ Two urine samples will be collected after P1. One will be collected shortly following the visit (the morning of the pickup of the air pumps and other environmental equipment). The second is intended to measure environmental exposures closer to the time of conception and will be collected the morning after a positive pregnancy test and frozen. Participants will be provided with shipping material to mail this sample to the repository.

⁴ Saliva kits will be left at the visit and adult participants directed to collect 3 samples each day for 2 days following the visit and store them in a freezer. For child participants, parents will be directed to collect 2 samples each day for 2 days following the visit and store them in a freezer.

⁵ Prior to the T3 clinic visit, participants will be sent a urine collection kit and a saliva collection kit. They will be directed to collect samples and freeze them and bring the samples to the clinic visit.

⁶ A breast milk kit will be given at the birth visit and milk will be collected at 1 month and mailed in. A breast milk kit will be sent after the 3 month phone call to mothers who are still nursing and the participant will mail the milk sample in. For mothers who are still nursing, a kit will be sent prior to the 6 month visit for collection prior to the visits. Milk will be picked up during the visit.

NCS PROTOCOL BIOSPECIMEN DETAILS (continued)
June 20, 2007 – Version 1.3

Simplified Summary of Cord Blood (mL) at Birth

	Pregpregnancy				Pregnancy							Birth				Postnatal										
	P1 Home	Materials left behind and picked up after P1 ¹	P 1 month (Phone)	P 2 months (Phone)	P 4 months (Phone)	T1-1st (Home)	T1-Prior (Home)	Materials left behind and picked up after T1 ¹	16-17 weeks (Phone)	T2 (Clinic)	T3 (Clinic)	Materials left behind and picked up after T3 ¹	36 weeks (Phone)	B1 Delivery (Hospital)	B2 Pre-discharge (Hospital)	Materials left behind and picked up after B2 ¹	1 month visit if needed (Home)	3 months (Phone)	6 month visit (Home)	Materials left behind and picked up after 6 mo. visit ¹	9 months (Phone)	12 month visit (Home)	Materials left behind and picked up after 12 mo. visit ¹	18 months (Phone)	24 months (Phone)	
Endocrine panel																										
Cortisol														X												
Cortisone														X												
Corticotropin releasing hormone														X												
Cortisol binding globulin														X												
CRH binding protein														X												
ACTH														X												
Reproductive																										
Estriol														X												
Estradiol														X												
Progesterone														X												
Infection/Inflammation/Biological																										
Hemoglobin/Hematocrit														X												
Cytokines/Interleukins														X												
Ig types and subtypes																										
Herpes simplex 1 & 2														X												
Hepatitis profile														X												
Toxoplasmosis (toxoplasma gondii)														X												
Varicella														X												
Rubella (IgM antibody)														X												
Syphilis														X												
IgE (cat, dog, cockroach, dust mite, fungi, mouse/rat urine)														X												
C-reactive protein (CRP)														X												
Homocysteine, fasting preferred														X												
RBC folate														X												
Thyroid (TSH and free t4)														X												
Antioxidant (vitamins A/E, carotenoids), fasting														X												
Vitamin C, fasting														X												
Alcohol, % carb deficient transferrin														X												
N3-N6 fatty acids														X												
Glucose metabolism																										
Fasting glucose														X												
HgbA1C														X												
Fasting insulin														X												
Insulin-like growth factor														X												
Chemicals																										
Metals: mercury, cadmium, lead														X												
Lipids, PCBs, organochlorine pesticides, PBDE, perfluorinated compounds (PFOA,PFOS)														X												
Genetic material																										
Cryopreserved PBMCs														X												
Genomic DNA														X												
Mitochondrial DNA														X												
RNA														X												
Other																										
Guthrie card														X												
TOTAL volume needed for specified analytes (mL)														60.7												
# of blood tubes proposed to be collected																										
Pre-screened Lavender for metals														1- 3 mL												
Red top														3-10 mL												
Lavendar EDTA														2-10 mL; 1-6mL												
ACD tube for PBMC collection														1-10 mL												
PaxGene RNA														1-2.5mL												
Gray top NaF														1-4mL												

¹ Activity is initiated at in-person visit and requires participant action after the visit (e.g., mail-in, self-collected urine sample, complete and mail self-administered questionnaire). Time frame for completion varies and is specific to each activity.

NCS PROTOCOL BIOSPECIMEN DETAILS (continued)
June 20, 2007 – Version 1.3

Simplified Summary of Blood (mL) by Visit from Mother (M), Child (C), and Father (F)

	Prepregnancy					Pregnancy							Birth				Postnatal									
	P1 Home	Materials left behind and picked up after P1 ¹	P 1 month (Phone)	P 2 months (Phone)	P 4 months (Phone)	T1-1st (Home)	T1-Prior (Home)	Materials left behind and picked up after T1 ¹	16-17 weeks (Phone)	T2 (Clinic)	T3 (Clinic)	Materials left behind and picked up after T3 ¹	36 weeks (Phone)	B1 Delivery (Hospital)	B2 Pre-discharge (Hospital)	Materials left behind and picked up after B2 ¹	1 month visit if needed (Home)	3 months (Phone)	6 month visit (Home)	Materials left behind and picked up after 6 mo. visit ¹	9 months (Phone)	12 month visit (Home)	Materials left behind and picked up after 12 mo. visit ¹	18 months (Phone)	24 months (Phone)	
Endocrine panel																										
Cortisol						M F	M F				M															
Cortisone						M	M				M															
Corticotropin releasing hormone						M	M				M															
Cortisol binding globulin						M	M				M															
CRH binding protein						M	M				M															
ACTH						M	M				M															
Reproductive																										
Estriol						M	M				M															
Estradiol						M	M				M															
Progesterone						M	M				M															
Infection/Inflammation/Biological																										
Hemoglobin/Hematocrit	M					M	M				M												C			
Cytokines/Interleukins	M					M	M				M															
Ig types and subtypes																										
Herpes simplex 1 & 2 (Ig)	M					M	M				M															
Hepatitis profile (A & B) medical records	M					M	M				M															
Toxoplasmosis (toxoplasma gondii) (Ig)	M					M	M				M															
Varicella (Ig)	M					M	M				M															
Rubella (IgM antibody)	M					M	M				M															
Syphilis (Ig)	M					M	M				M															
IgE (cat, dog, cockroach, dust mite, fungi, mouse/rat urine)	M					M	M				M															
CRP	M					M	M				M															
Heat shock proteins	M					M	M				M															
Homocysteine						M	M				M															
RBC folate						M	M				M															
Thyroid (TSH and free t4)	M					M	M				M															
Antioxidant (vitamins A/E, carotenoids)						M	M				M															
Vitamin C						M	M				M															
Vitamin D (25OH)						M	M				M												C			
Alcohol, % carb deficient transferrin	M					M	M				M			M												
Omega FA (N3-N6)											M															
Glucose metabolism																										
Glucose (fasting)											M															
HgbA1C	M					M	M																			
Insulin (fasting)											M															
Insulin-like growth factor											M															
Chemical exposures																										
Metals: Mercury, cadmium, lead	M					M F	M F							M									C			
Lipids, PCBs, organochlorine pesticides, PBDE, perfluorinated compounds (PFOA, PFOS)	M					F	F																C			
Combination of dioxins/furans and all other chemicals (excluding metals)						M	M				M			M												
Genetics																										
Cryopreserved PBMCs						F	F				M															
Genomic DNA [^]						M F	M F																			
Mitochondrial DNA						M F	M F																			
RNA																										
Other																										
Guthrie card, heel stick														C												

TOTAL volume needed for specified analytes (mL)

Mother	26.2					42.6	42.6					56.9					33.5									
Father						24.2	24.2																			
Child																								13.6*		

of blood tubes proposed to be collected

Pre-screened Lavender for metals	1-3 mL					1-3 mL M, F	1-3 mL M, F										1-3 mL									1-3 mL
Red top	3-10 mL					3-10 mL M 1-10 mL F	3-10 mL M 1-10 mL F				3-10 mL						3-10 mL									2-5 mL
Lavendar EDTA	1-10 mL					2-10 mL M 1-10 mL F	2-10 mL M 1-10 mL F				2-10 mL															1-6 mL
ACD tube for PBMC collection						1-10 mL F	1-10 mL F				1-10 mL															
PaxGene RNA											1-2.5 mL															
Gray top NaF											1-4 mL															

¹ Activity is initiated at in-person visit and requires participant action after the visit (e.g., mail-in, self-collected urine sample, complete and mail self-administered questionnaire). Time frame for completion varies and is specific to each activity.

*A complete list of analytes for the child at 12 months have not been confirmed. A 6 mL Lavender tube is being proposed to be collected to allow for future determination of analytes.

[^]Saliva collection for DNA will be a backup if participants refuse blood draw.

NCS PROTOCOL BIOSPECIMEN DETAILS (continued)
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Summary of Breast Milk (mL) by Visit

	Prepregnancy				Pregnancy							Birth				Postnatal									
	P1 Home	Materials left behind and picked up after P1 ¹	P 1 month (Phone)	P 2 months (Phone)	P 4 months (Phone)	T1-1st (Home)	T1-Prior (Home)	Materials left behind and picked up after T1 ¹	16-17 weeks (Phone)	T2 (Clinic)	T3 (Clinic)	Materials left behind and picked up after T3 ¹	36 weeks (Phone)	B1 Delivery (Hospital)	B2 Pre-discharge (Hospital)	Materials left behind and picked up after B2 ¹	1 month visit, if needed* (Home)	3 months (Phone)	6 month visit (Home)	Materials left behind and picked up after 6 mo. visit ¹	9 months (Phone)	12 month visit (Home)	Materials left behind and picked up after 12 mo. visit ¹	18 months (Phone)	24 months (Phone)
Antioxidants: Vitamins C/E, beta-carotene																	M	M		M					
Phytoestrogens																	M	M		M					
Component: lipid, proteins, carbohydrates, enzymes, immunoglobulins, minerals, vitamins, cytokines, hormones																	M	M		M					
Chemical exposures																									
Dioxins/furans; organochlorine pesticides; PCBs																	M	M		M					
Pesticides																	M	M		M					
PBDE																	M	M		M					
Perchlorate, iodide, thiocyanate, nitrate																	M	M		M					
Manganese																	M	M		M					
Phenols-bisphenol A and parabens																	M	M		M					
TOTAL volumes (mL)																	80-100 ml	80-100 ml	80-100 ml						

¹ Activity is initiated at in-person visit and requires participant action after the visit (e.g., mail-in, self-collected urine sample, complete and mail self-administered questionnaire). Time frame for completion varies and is specific to each activity.

* Breast milk instructions and supplies will be given at birth, but the first self-collection will occur around 1 month postnatal.

NCS PROTOCOL BIOSPECIMEN DETAILS (continued)
June 20, 2007 – Version 1.3

Simplified Summary of Urine (mL) From Mother (M), Father (F), and Child (C)

	Prepregnancy				Pregnancy						Birth				Postnatal											
	P1 Home	Materials left behind and picked up after P1 ¹	P 1 month* (Phone)	P 2 months (Phone)	P 4 months (Phone)	T1-1st (Home)	T1-Prior (Home)	Materials left behind and picked up after T1 ¹	16-17 weeks (Phone)	T2 (Clinic)	T3 (Clinic)	Materials left behind and picked up after T3 ¹	36 weeks (Phone)	B1 Delivery (Hospital)	B2 Pre-discharge (Hospital)	Materials left behind and picked up after B2 ¹	1 month visit if needed (Home)	3 months (Phone)	6 month visit (Home)	Materials left behind and picked up after 6 mo. visit ¹	9 months (Phone)	12 month visit (Home)	Materials left behind and picked up after 12 mo. visit ¹	18 months (Phone)	24 months (Phone)	
Illicit drug panel		M	M					M				M		M											C	
Cortisol								M																		
Preganancy tests		M	M																							
PCR for chlamydia		M	M					M				M														
PCR for gonorrhea		M	M					M				M														
Chemical exposures																										
Atrazine + pyrethroids + OPs + carbamates + ETU/PTU/EBC		M	M					M F				M		M												C
Phthalates and bisphenol A/nonyl phenol		M	M					M F				M		M												C
Halogenated phenols(PCP)		M	M					M F				M		M												C
Perchlorate		M	M					M F				M		M												C
PAH		M	M					M F				M		M												C
Iodine		M	M					M F				M		M												C
Urine metals (multi-element panel, includes Cd, As, etc.)		M	M					M F				M		M												C
Mercury		M	M					M F				M		M												C
Creatinine		M	M					M F				M		M												C
Cotinine		M	M					M F				M		M												C
Phytoestrogens		M	M					M F				M		M												C
TOTAL volume needed for specified analytes (mL)																										
Mother		33.5	33.5					33.5				33.5		31.5												
Father								31.5																		
Child																										31.0

¹ Activity is initiated at in-person visit and requires participant action after the visit (e.g., mail-in, self-collected urine sample, complete and mail self-administered questionnaire). Time frame for completion varies and is specific to each activity.

* P 1 month urine sample: A first morning void urine will be collected the morning after a positive pregnancy test.

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Vaginal Swab Summary for Mother (M)

	Prepregnancy					Pregnancy								Birth				Postnatal								
	P1 Home	Materials left behind and picked up after P1 ¹	P 1 month (Phone)	P 2 month (Phone)	P 4 month (Phone)	T1-1st (Home)	T1-Prior (Home)	Materials left behind and picked up after T1 ¹	16-17 weeks (Phone)	T2 (Clinic)	T3 (Clinic)	Materials left behind and picked up after T3 ¹	36 weeks (Phone)	B1 Delivery (Hospital)	B2 Pre-discharge (Hospital)	Materials left behind and picked up after B2 ¹	1 month visit if needed (Home)	3 months (Phone)	6 month visit (Home)	Materials left behind and picked up after 6 mo. visit ¹	9 months (Phone)	12 month visit (Home)	Materials left behind and picked up after 12 mo. visit ¹	18 months (Phone)	24 months (Phone)	
Vaginal swabs																										
pH											M															
Bacterial vaginosis	M					M	M				M															
Antibodies	M					M	M				M															
Cytokines	M					M	M				M															
Metalloproteinase	M					M	M				M															

¹ Activity is initiated at in-person visit and requires participant action after the visit (e.g., mail-in, self-collected urine sample, complete and mail self-administered questionnaire). Time frame for completion varies and is specific to each activity.

NCS PROTOCOL BIOSPECIMEN DETAILS (continued)
June 20, 2007 – Version 1.3

Placenta, Umbilical Cord, and Meconium Summary at Birth

	Prepregnancy				Pregnancy							Birth				Postnatal										
	P1 Home	Materials left behind and picked up after P1 ¹	P 1 month (Phone)	P 2 month (Phone)	P 4 month (Phone)	T1-1st (Home)	T1-Prior (Home)	Materials left behind and picked up after T1 ¹	16-17 weeks (Phone)	T2 (Clinic)	T3 (Clinic)	Materials left behind and picked up after T3 ¹	36 weeks (Phone)	B1 Delivery (Hospital)	B2 Pre-discharge (Hospital)	Materials left behind and picked up after B2 ¹	1 month visit, if needed (Home)	3 months (Phone)	6 month visit (Home)	Materials left behind and picked up after 6 mo. visit ¹	9 months (Phone)	12 month visit (Home)	Materials left behind and picked up after 12 mo. visit ¹	18 months (Phone)	24 months (Phone)	
Placenta																										
Antibodies and cytokines														M												
Pathology														M												
Chemical contaminants														M												
Umbilical cord																										
Antibodies and cytokines														M												
Pathology														M												
Chemical contaminants														M												
Meconium																										
Cotinine															C											
Organophosphate metabolites															C											

¹ Activity is initiated at in-person visit and requires participant action after the visit (e.g., mail-in, self-collected urine sample, complete and mail self-administered questionnaire). Time frame for completion varies and is specific to each activity.

NCS PROTOCOL BIOSPECIMEN DETAILS (continued)
June 20, 2007 – Version 1.3

Simplified Summary of Hair, Nail, and Saliva Collection From Mother (M), Father (F), and Child (C)

	Prepregnancy				Pregnancy							Birth				Postnatal										
	P1 Home	Materials left behind and picked up after P1 ¹	P 1 month (Phone)	P 2 month (Phone)	P 4 month (Phone)	T1-1st (Home)	T1-Prior (Home)	Materials left behind and picked up after T1 ¹	16-17 weeks (Phone)	T2 (Clinic)	T3 (Clinic)	Materials left behind and picked up after T3 ¹	36 weeks (Phone)	B1 Delivery (Hospital)	B2 Pre-discharge (Hospital)	Materials left behind and picked up after B2 ¹	1 month visit if needed (Home)	3 months (Phone)	6 month visit (Home)	Materials left behind and picked up after 6 mo. visit ¹	9 months (Phone)	12 month visit (Home)	Materials left behind and picked up after 12 mo. visit ¹	18 months (Phone)	24 months (Phone)	
Hair																										
Cotinine	M					M F	M F				M								C			C				
Total Hg (assumed to be organic ethyl.	M					M F	M F				M								C			C				
Nail																										
Organic Hg (ethyl, methyl)						F	F				M															
Hg inorganic						F	F				M															
Saliva*																										
Cortisol								M				M								M F			C			

¹ Activity is initiated at in-person visit and requires participant action after the visit (e.g., mail-in, self-collected urine sample, complete and mail self-administered questionnaire). Time frame for completion varies and is specific to each activity.

* Saliva kits will be left at the visit and adult participants directed to collect 3 samples each day for 2 days following the visits and store them in a freezer. For child participants, parents will be directed to collect 2 samples each day for 2 days following the visit and store them in a freezer.