Sister Study Baseline Specimen Collection and Processing Summary

Baseline Specimen Collection

Sister Study specimens were collected at study participants' homes or a mutually agreed upon alternate site, such as a doctor's office. Participants were sent a kit for collecting first morning void urine, toenails, and household dust. Samples, along with anthropometric and blood pressure measurements, were collected by examiners from a national in-home phlebotomy service.

Blood collection: Participants were instructed to fast for at least eight hours prior to their blood draw. The examiner drew the blood as shown in the table below, and retrieved the urine and environmental samples. Each participant had approximately 45 ml of blood collected into six BD Vacutainer® tubes, including two EDTA tubes, two serum tubes and two ACD-B tubes.

TUBE COLOR/TOP	TUBE TYPE	DRAW ORDER	PACKING	SPECIAL HANDLING
Red	10 ml	(1)	Cold *	*Only while in transit back to exam office before processing. Invert
Red	10 ml	(2)	Cold *	*Only while in transit back to exam office before processing. Invert
Purple	EDTA 10 ml	(3)	Ambient	Invert
Yellow	ACD-B 6 ml	(4)	Ambient	Invert
Tan	EDTA 3 ml	(5)	Ambient	Invert
Yellow	ACD-B 6 ml	(6)	Ambient	Invert

Saliva collection: In the event that a blood sample could not be collected due to an unsuccessful phlebotomy, participants were asked to do a saliva collection using an Oragene™ DNA self-collection saliva kit. Although this will have its limitations compared to blood, it will provide a DNA source for genetic analyses.

Urine collection: On the morning of the examiner visit, participants collected approximately 60 ml of their first urine of the day in a collection cup that was pre-tested for phthalates.

Toenail collection: After removing any nail polish, the participant took a clipping from each toenail on both feet. Big toenails were stored separately from other toenails in provided envelopes.

Dust collection: Three door frames from three different rooms in the house were swiped for dust resulting in six dirty swabs from three different locations.

Baseline Specimen Processing

Serum and blood clot: Two red-top tubes were centrifuged in the field immediately after collection in order to isolate the serum from the red cells as soon as possible. The serum was transferred to a standard transport tube for return to the lab. Upon arrival, the serum was stored in approximately fifteen 0.5 ml CryoBioSystem™ (CBS™) straws in liquid nitrogen vapor phase. One blood clot was stored in a cryovial.

Plasma and EDTA whole blood: A 10.0 ml EDTA BD Vacutainer® tube (purple top) provided whole blood, plasma, and dried blood. A 1.0 ml aliquot of whole blood was stored in a cryovial and two types of dry blood storage cards were spotted and stored (60 µl per spot). One was a treated card chemically impregnated to lyse cells and stabilize DNA and the other was an untreated card. The remaining whole blood was centrifuged and the plasma was isolated and stored in 0.5 ml CBS™ straws in liquid nitrogen vapor phase. A 3.0 ml EDTA BD Vacutainer® tube (tan top) was stored in the original Vacutainer® tube at -20°C for future analysis of metals, trace elements, and environmental contaminants.

ACD-B whole blood: One of the ACD-B Vacutainer® tubes was aliquotted into six 1.0 ml cryovials and cryopreserved with 10% DMSO using a programmable freezer. Fifteen percent of the time, this ACD-B tube was selected for lymphocyte isolation as an alternative protocol. Selection was based on an algorithm that predetermined a high risk group based on age of enrollment and the affected sister's age at diagnosis plus randomly selected controls. The second ACD-B tube was aliquotted into one 4.5 ml cryovial and two 1.0 ml cryovials and stored in liquid nitrogen vapor phase.

Urine: Immediately upon receipt at the Sister Study lab, a basic chemistry urinalysis was performed to measure protein, creatinine, blood, leukocytes, nitrite, glucose, ketone, pH, and specific gravity. Urine was aliquotted into twenty 0.5 ml CBS™ straws, five 1.0 ml vials, and one 3.6 ml vial. Urine straws were stored in liquid nitrogen vapor phase and vials were stored in -80°C mechanical freezers.

Specimen Storage Summary per Sister Study Participant				
Material type	**Additive**	**Container**	**Volume**	**Final Storage Temp.**
Blood clot	None	Vial	1	-80C/LN
Lymphocytes	ACD	Cryopreserved vial	1-2 vials, ~3 million cells/vial	LN
Plasma	EDTA	Straws	9 X 0.5 ml	LN
Serum	None	Straws	17 X 0.5 ml	LN
Whole blood	ACD	Vial	1 X 4.5 ml	-80C/LN

Specimen Storage Summary per Sister Study Participant				
Material type	**Additive**	**Container**	**Volume**	**Final Storage Temp.**
			2 X 1.0 ml	LN
		Cryopreserved vial	6 X 1.0 ml	LN
Whole blood	EDTA	Vacutainer	1 X 3.0 ml	-20C
		Vial	1 X 1.0 ml	-80C
		Filter cards	2	Ambient
Urine	None	Straws	20 X 0.5 ml	LN
		Vial	1 X 3.6 ml	-80C
			5 X 1.0 ml	-80C
Toenails	None	Envelope	2	Ambient
Household dust	None	Alcohol swabs in sealed plastic bag	6 swabs	-20C