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1. Biospecimen collection by clinic visit.

HCHS/SOL collected fasting blood specimens and urine at all three clinic visits. Specifically, 60mL of blood in Visit 1, 55.5 mL blood in Visit 2, and 50.5 mL blood in Visit 3. Detailed information on what was collected each visit and amount used for participants' report are included in **Tables 1 and 2**.

	Visit 1 (200	8-2011)	Visit 2 (2014-2017)		Visit 3 (2020-2024)	
Sample Type	Total # of vials	Volume /vial	Total # of vials	Volume/ vial	Total # of vials	Volume /vial
Serum	14	400 uL	14	500 uL	15	500 uL
Citrate Plasma	7	500 uL	2	500 uL	2-3	500 uL
EDTA Plasma	15	500 uL	13	500 uL	13	500 uL
Post-OGTT EDTA Plasma	2	500 uL	2	500 uL	-	-
Neutral Urine	2	900 uL	2	1500 uL	3	1500 uL
Acid Urine	1	1500 uL	1	1500 uL	1	1500 uL
Alkaline Urine	1	1500 uL	1	1500 uL	1	1500 uL
Packed Cells	1	4 mL	-	-	1	4 mL
Stock DNA Vials	1	200 ug	1	29 ug	-	-
		ave		ave		
Intermediate DNA Dilutions	1	50 ug	-	-	-	-
Paxgene Whole Blood	1-2	2.5 mL	-	-	-	-
RNA	3	1.2 ug	-	-	-	-
		ave				
EDTA Whole Blood	2	2.5 mL	2	1.0 mL	2	1.0 mL

Table 1. Biosample type collection by clinic visit, HCHS/SOL.

Table 2. Biospecimen volume used for immediate feedback report testing by sample type and clinic visit, HCHS/SOL.

Sample Type	Visit 1 (2008-2011) Volume	Visit 2 (2014-2017) Volume	Visit 3 (2020-2024) Volume
Serum	1 mL	1 mL	250 uL
EDTA Plasma	200 uL	250 uL	250 uL
Post-OGTT EDTA Plasma	200 uL	250 uL	-
Neutral Urine	1.5 mL	1 mL	-
EDTA Whole Blood	1 mL	1 mL	1.5 mL

2. Biorepository availability by storage location and by type of Visit

Tables 3A, 3B and 3C include the number of samples collected by visit. Visit 1 included 17,382 collections (n=16,415 participants), Visit 2 included 13,023 collections (n=11,623 participants), and Visit 3 included 10,054 collections (n=9,090 participants). Note that these totals include blind duplicates, as well as repeat collections at Visit 1.

Sample Type	Total # of Vials	# of Sample Collections	Storage Location	Total # of Vials	# of Sample Collections	Storage Location
Serum	7	17,332	ARDL	7	17,331	OFF SITE
Citrate Plasma	4	17,260	ARDL	3	17,224	OFF SITE
EDTA Plasma	7	17,310	ARDL	8	17,305	OFF SITE
POST OGTT Plasma	1	14,440	ARDL	1	14,407	OFF SITE
Neutral Urine	2	17,291/17,270	ARDL	-	-	-
Acid Urine	-	-	-	1	17,294	OFF SITE
Alkaline Urine	-	-	-	1	17,291	OFF SITE
DNA	3	17,137	ARDL	-	-	-
Packed Cells	-	-	-	1	17,022	OFF SITE
Paxgene Whole Blood	1/0.5	9,368/7,743	ARDL	1	16,216	OFF SITE
RNA	2	7,743	ARDL	-	-	-
EDTA Whole Blood	1	17,337	ARDL	1	17,328	OFF SITE

Table 3A. Biorepository by storage location at HCHS/SOL Visit 1

18 TOTAL FREEZERS, 9 ONSITE and 9 AT BACKUP LOCATION

Table 3B. Biorepository by storage location at HCHS/SOL Visit 2

Sample Type	Total # of Vials	# of Sample Collections	Storage Location	Total # of Vials	# of Sample Collections	Storage Location
Serum	8	12,932	ARDL	6	12,879	OFF SITE
Citrate Plasma	1	12,847	ARDL	1	12,847	OFF SITE
EDTA Plasma	6	12,908	ARDL	7	12,908	OFF SITE
POST OGTT Plasma	1	9,612	ARDL	1	9,612	OFF SITE
Neutral Urine	2	12,958	ARDL	-	-	-
Acid Urine	-	-	-	1	12,957	OFF SITE
Alkaline Urine	-	-	-	1	12,955	OFF SITE
DNA	1-2	5,763	ARDL	-	-	-
RNA	1 (LABiomed)	298	ARDL	-	-	-
EDTA Whole Blood	1-2	6,288/6,647	ARDL	1	12,877	OFF SITE

9 TOTAL FREEZERS, 5 ONSITE and 4 AT BACKUP LOCATION

Table 3C. Biorepository by storage location at HCHS/SOL Visit 3

Sample Type	Total # of vials	# of Sample Collections	Storage Location	Total # of Vials	# of Sample Collections	Planned Location		
Serum	8	10,054	ARDL	7	10,054	OFF SITE		
Citrate Plasma	1-2	10,054	ARDL	1	10,054	OFF SITE		
EDTA Plasma	7	10,050	ARDL	6	10,050	OFF SITE		
Neutral Urine	3	10,054	ARDL	-	-	-		
Acid Urine	-	-	-	1	10,054	OFF SITE		
Alkaline Urine	-	-	-	1	10,054	OFF SITE		
Packed Cells	1	10,054	ARDL	-	-	-		
EDTA whole blood	2	10,054	ARDL	-	-	-		

7 TOTAL FREEZERS, ALL CURRENTLY ON SITE, WORKING ON DIVIDING THE INVENTORY TO SEND APPROXIMATELY HALF OF THE SAMPLES OFF SITE.

Table 4 shows the percentage of incomplete sample collections by clinic visit. A sample collection is considered complete when there was enough blood collected for all required aliquots to be prepared. Overall, the percentage of incomplete samples is 9.2% at baseline, 4% at Visit 2, and 6.7% at Visit 3. The percentage of incomplete samples varies from 2.0% to 12.2% by field center and visit. HCHS/SOL protocols were established to collect 49, 37 and 39 aliquots at Visits 1, 2 and 3 respectively. Among incomplete sets, the average missing aliquots is low and ranges from 1.8 to 12.7.

		Total Collections	Collections Complete	Collections Incomplete	Percent Incomplete	Total Aliquots	Total Aliquots Missing	Number of Aliquots per Complete Set	Average Missing Aliquots per Incomplete Set
Baseline (2008- 2011)	Total	17382	15790	1592	9.2	850018	5329	49	3.4
Visit 2 (2014-	Frozen	13039	12516	523	4.0	449557	2969	35	5.7
2017)	Whole blood	13023	12973	50	0.4	26046	91	2	1.8
Visit 3 (2020- 2023)	Total	10054	9377	677	6.7	390857	5121	39, 38 after 8/11/22*	7.6

Table 4. Biorepository by Study Visit, HCHS/SOL.

*Started study with a 4.5 mL sodium citrate tube collection and 3 aliquots. Due to the COVID pandemic causing numerous supply chain shortages, often only 2.7 mL sodium citrate tube was available commercially, causing more incomplete collections. As time went on, we learned that the manufacturers were discontinuing the 4.5 mL tube and it would not be coming back. On 8/11/22, we switched the aliquot schema to 2 aliquots.

3. Biorepository Impact by Ancillaries.

A Biorepository Impact Statement is prepared for each proposal submitted to the Ancillary Study Committee. The amount of sample requested is reviewed by the Laboratory Committee for appropriateness. The Ancillary Studies policy states that up to 250 uL of sample (plasma/serum) can be given for any single ancillary study. There have been a few exceptions in the past when there has been adequate scientific justification for a higher volume. The Lab has also established a Biorepository Reserve policy to ensure that the study does not run out of biospecimens for any individual participants. This consists of a "Long Term Biorepository Reserve" (LTBR) that includes biospecimens (1-2 aliquots per sample type) for use in future assays that cannot be predicted using current technologies and will not be used until 2034, as well as a "Restricted Use Biorepository Reserve" (RUBR) that includes specimens that have a limited number of aliquots in the HCHS/SOL biorepository. Biospecimens that are part of the RUBR will receive additional scrutiny before approval. It will likely be a provisional approval, conditional on the logistics of sample retrieval and the use of samples by other approved ancillary studies. If more than one study that is approved for these samples receive funding, the laboratory will work with the coordinating center and the Principal Investigators of the ancillary studies to ensure everyone has the maximum number of samples possible, given the afore-mentioned logistical considerations.

At the request of the OSMB committee, we have recently updated the LTBR to include DNA samples as well. For V1 and V3 samples, the second dilution vial of ~50 ug will be designated as LTBR. A packed cell aliquot for DNA extraction was not collected as part of the V2 core study, so no specific vial will be designated as LTBR. We do have two small (1 mL) V2 EDTA whole blood aliquots available for DNA extraction if resources are available. We will monitor use of these resulting DNA vials and make sure they never go lower than 5 ug.

As of 7/15/2024, biospecimens have been provided to 44 ancillary studies. Biorepository volume used per sample type and clinic visit is shown on **Table 5**. Sample size and eligibility criteria vary by ancillary study. Most studies use a relatively small number of samples compared to the total available in the biorepository so there is relatively little overlap. The lower end of range of remaining volume assumes all studies utilized the same sample list which is unlikely. Most sample collections are likely to be in the upper end of the range.

Sample Type	Visit	Study	Date	Amount (uL)	Number of
			Completed	(ug for DNA)	samples (n)
Citrate plasma	1	H. Pylori Serology (pilot)	April 2017	100	39
Citrate plasma	1	H. Pylori Serology	Nov 2017	100	16,202
Citrate plasma	1	Hemostasis Phenotypes	In progress	250	12,600
DNA	1	OLA	May 2013	2.5	13,586
DNA	1	Matise_PAGEII/CALiCo II	Dec 2014	1.875	1,074
DNA	1	Addendum to PAGEII/CALiCo II	Sept 2015	1	282
DNA	1	SOL WGS and Metabolomics	Oct 2016	2	4,250
DNA	1	SOL TOPMED	Feb 2017	2.5	2,266
DNA	1	SOL WGS and Metabolomics	April 2017	2	267
DNA	1	SOL TOPMED	Oct 2017	2.5	1,739
DNA	1	Spirituality and Health	Nov 2019	2.4	1,000
DNA	1	SOL-INCA DNAm	March 2021	1.6	2,880
DNA	1	APOE	Dec 2021	0.625	1,585
DNA	1	Social Stress Epigenetics	Jan 2022	1	1,000
DNA	1	Epigenetics of Preterm Birth	Feb. 2024	1	120
DNA	1	CALICO I	Jan 2012	3	12.667
DNA	1	CaLiCo II	Dec 2014	2	12,917
DNA	1	TOPMed DNAm	In progress	1	9,000
EDTA plasma	1	Wolf-Meta	April 2016	150	1,206
EDTA plasma	1	FGF23-Sleep	April 2016	155	1,085
EDTA plasma	1	FGF23-Sleep	April 2016	310	121
EDTA plasma	1	H. Pylori Serology (pilot)	April 2017	100	39
EDTA plasma	1	Pesticides	Feb 2018	1000	2,350
EDTA plasma	1	SOL AS2015.23	Jan 2022	250	1,068
EDTA plasma	1	Eicosanoid Metabolites	Aug 2022	500	16,415
EDTA plasma	1	SOL INCA2	March 2023	250	6,503
RNA	1	TOPMed RNA Seq	June 2023	1 aliquot	7,644
Serum	1	Wolf-Meta	April 2016	50	1,206
Serum	1	Diet and Lifestyle and Diabetes Risk	May 2016	100	40
Serum	1	SOL WGS and Metabolomics	Sept 2016	500	4,250
Serum	1	SOL WGS and Metabolomics	April 2017	500	267
Serum	1			500	-
Serum	1	Pesticides GOLD	June 2018 Aug 2020	400	2,350 2,319
	1			400	
Serum	1	Metabolomics and Cardiac Function	Aug 2020		1,350
Serum	1	TOPMed Metabolomics	March 2021	250 400	17,146 7,733
Serum			April 2024		,
Serum	1	Tobacco Biomarkers	In progress	200 500	2,000 2,000
Urine, neutral	<u>1</u> 1	Tobacco Biomarkers	In progress March 2024		,
Urine, neutral		Food Based Biomarkers		500	206
Urine, neutral	1	Food Based Biomarkers	In progress	500	60
DNA	2	SOL-INCA DNAm	March 2021	1.6	2,880
DNA	2	APOE	Dec 2021	0.625	480
DNA	2	Social Stress Epigenetics	Jan 2022	1	1,000
DNA	2	TOPMed DNAm	In progress	1	4,000
DNA	2	DNAm and Socioenvironmental Exposure	In progress	1	3,000
EDTA plasma	2	SOL INCA2	March 2022	250	6,541
EDTA plasma	2	Eicosanoid Metabolites	Aug 2022	500	12,456
EDTA plasma	2	SOL INCA2	March 2022	250	6,041
Serum	2	Pesticides	June 2018	50	470
Serum	2	GOLD	Aug 2020	500	859
Serum	2	Metabolomics and Cardiac Function	Aug 2020	500	1,000

Table 5. Biospecimens utilized by Ancillary Studies.

Sample Type	Visit	Study	Date Completed	Amount (uL) (ug for DNA)	Number of samples (n)
Serum	2	Neighborhood Greenness	April 2023	50	6,115
Serum	2	TOPMed Metabolomics	April 2024	500	4,298
EDTA plasma	3	VIDA	In progress	50	2,600
EDTA plasma	3	SOL INCA-AD	In progress	250	6,987
Serum	3	C4R	Oct 2022	250	3,580
Serum	3	C4R	March 2024	250	3,012
Serum	3	Sexual and gender minorities	June 2024	50	1,284
Serum	3	PASOS	In progress	50	4,500
Serum	3	Asthma Epigenomics	In progress	500	700
Neutral urine	3	PASOS	In progress	50	4,500
DNA	SOL Youth	Genetics of Obesity	Sept 2019	0.6	1,410
DNA	SOL Youth	Social Stress Epigenetics	March 2023	1.4	1,114
Serum	SOLNAS	Dietary Patterns and CVD	Oct 2019	250	462
Serum	SOLNAS	Tasevska SOLNAS Ancillary Study	In progress	40	578
Spot urine	SOLNAS	Arsenic Study	July 2016	500	88
Spot urine	SOLNAS	Food Based Biomarkers	Oct 2022	500	464