

## Biospecimen Protocol: Lessons Learned from the Initial Phase of the NCS Vanguard Study

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For the initial phase (Phase 0) of the NCS Vanguard Study, the Coordinating Center (CC) developed standard operating procedures for collection, processing, storage and shipping of biospecimens including venous blood, hair, nails, saliva vaginal swabs and slides, urine, cord blood, placenta, meconium, dried blood spots and breast milk. The CC trained and certified data collectors from seven Screening Centers (SCs) and monitored all biospecimen and related data collection activities. When problems were identified, the CC investigated causes and when appropriate, developed alternative procedures. Operational issues were documented to be evaluated in conjunction with the results of the analysis operational data and biospecimens to assess the acceptability and feasibility of Phase 0 NCS biospecimen measures. Response rates were determined for each biospecimen measure as an indicator of acceptability and feasibility. Laboratory test results were compared to normal reference ranges for selected biomarkers to evaluate quality and suitability of biospecimens and the technical performance of procedures.

A variety of valuable lessons were learned regarding operations at the SCs, the repository and the laboratories. 1) The cord blood collected in a bag with liquid CPD was not suitable for a variety of critical analysis. 2) The P100 tubes separators did not function properly upon centrifugation of the tubes according to manufacturer's instructions. 3) Saliva samples contained excessive amounts of particulate matter necessitating extensive cleaning prior to analysis. 4) The repository procedures for aliquotting breast milk introduced perchlorate contamination. 5) The standard procedure for shipping placentas was not adequate for all geographic locations and seasons.

SCs achieved high success (approximately 80 to 100%) in completion of sample collection for nearly all specimen types at all visits. Exceptions were 2-day saliva (66%), paternal hair (63%), and breast milk or formula (70%).

The early results of laboratory analysis are encouraging. Three biomarkers of diabetes - glucose, hemoglobin A1c (HbA1c) and insulin, and two biomarkers of stress, ACTH and cortisol - were analyzed in pregnant women. A small percentage had elevated levels of serum glucose and HbA1c consistent with development of gestational diabetes in pregnancy. For serum insulin, 27% of NCS subjects had measured values above the reference range. For plasma ACTH, 26% of samples had values above the normal reference range for non-pregnant females, consistent with higher levels of ACTH found in pregnant women. Cortisol measured in saliva is expected to have elevated levels in morning samples, and low levels in evening samples. This was confirmed in the tested NCS subjects. The mean for morning and evening samples was 0.66 ng/mL and 0.15 ng/mL respectively. However, for both morning and evening samples, about half of the samples were above the normal reference range for non-pregnant levels consistent with expected higher levels in pregnant women.