Evaluation of Biological Specimen Protocols in the Vanguard Study (VS).

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Background: We evaluated biological specimen collection protocols and Repository processing by measuring a panel of environmental and nutritional analytes in samples collected by each VS center. Collection materials had been prescreened or evaluated by lab research staff and determined not to contain interfering substances that would contaminate the specimens. We planned to investigate anomalous results and use these findings to modify protocols, as appropriate. Analyte values were compared with U.S. population reference ranges from the National Health and Nutrition Examination Survey (NHANES).

Methods: Prenatal blood and urine, cord blood, and breast milk specimens collected from mothers and infants at each VS center and then shipped to the central Repository, were sent to the DLS at CDC. Specimens were collected and transported according to VS protocols. Pre-screened collection containers were used for blood and urine metals. Samples were collected using kits prepared by NCS and then shipped to the Repository where aliquots were prepared and frozen at -150°C according to protocols. Blood metals tubes and aliquots for specific analytes were shipped on dry ice to the DLS, where measurements were completed using analytical methods developed and used for the NHANES.

Results: Analytes included persistent and non-persistent chemicals, pesticides, and metals. Environmental chemical results in prenatal urine and serum generally were within reference ranges for U.S. females in NHANES. In breast milk specimens, 9% had detectable bisphenol A levels. Too few infant urine specimens were available for analysis because the timing coincided with an interruption of specimen collection in the VS.

The collection method for cord blood rendered specimens unsuitable for analysis, and as a result, the protocol has been modified. Anomalous perchlorate results for several breast milk aliquots were investigated and determined to have resulted from inadvertent contamination by perchloric acid during the milk aliquotting process. Modifications have been made to the Repository's aliquotting procedure. Planned infant urine collection using diaper inserts or diapers was changed to use of adhesive collection bags after it was determined that diaper materials would contaminate urine with various chemicals, including perchlorate and bisphenol A.

Conclusion: Our approach provided actionable results for validating and improving biological specimen protocols, and it was an invaluable step prior to implementing protocols across additional study centers. Failure to conduct a limited feasibility study such as this could risk wasted efforts in specimen collection and unwitting introduction of external contaminants into specimens. Use of pre-screened collection containers for metals, and for other analytes, selecting collection containers that would not be a source of chemical contamination are essential planning activities prior to collecting biological specimens.