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Planned Variation in Preanalytical Conditions to Evaluate Biospecimen Stability in the National Children’s Study (NCS)

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Abstract

BACKGROUND—Preanalytical conditions encountered during collection, processing, and storage of biospecimens may influence laboratory results. The National Children’s Study (NCS) is a planned prospective cohort study of 100,000 families to examine the influence of a wide variety of exposures on child health. In developing biospecimen collection, processing, and storage procedures for the NCS, we identified several analytes of different biochemical categories for which it was unclear to what extent deviations from NCS procedures could influence measurement results.

METHODS—A pilot study was performed to examine effects of preanalytic sample handling conditions (delays in centrifugation, freezing delays, delays in separation from cells, additive delay, and tube type) on concentrations of eight different analytes. 2,825 measurements were made to assess 15 unique combinations of analyte and handling conditions in blood collected from 151 women of childbearing age (20 individuals per handling condition).

RESULTS—The majority of analytes were stable under the conditions evaluated. However, levels of plasma interleukin-6 and serum insulin were decreased in response to sample centrifugation delays of up to 5.5 hours post collection ($P<0.0001$). In addition, delays in freezing centrifuged plasma samples (comparing 24, 48 and 72 hours to immediate freezing) resulted in increased levels of adrenocorticotrophic hormone ($P=0.0014$).

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CONCLUSIONS—Determining stability of proposed analytes in response to preanalytical conditions and handling helps to ensure high-quality specimens for study now and in the future. The results inform development of procedures, plans for measurement of analytes, and interpretation of laboratory results.

Keywords

ACTH; Biospecimen stability; CDT; estradiol; free T₄; IGF-1; IL-6; Insulin; vitamin C; Preanalytical variation

Introduction

The NCS is a planned prospective cohort study of 100,000 families designed to examine the influence of the environment on child health and development. Biospecimens proposed for collection include blood (whole blood, serum, plasma, buffy coat, and blood spots), urine, breast milk, vaginal swabs, hair, nails, meconium, and saliva. Measurements of analytes in these biological matrices will address key research questions and facilitate assessments of exposures and outcomes (1).

Differences in preanalytical sample handling conditions—such as delays in processing blood specimens, delays in freezing specimens for long-term storage, and length of specimen storage—may alter the properties of a biospecimen and potentially influence study results (2-9). Delays in centrifugation of blood specimens for isolation of serum or plasma can result in prolonged contact with cells and possible degradation of a particular analyte (e.g., through proteolytic activity), reduction of analyte levels through metabolism (e.g., glycolysis), or diffusion of a particular analyte out of blood cells (e.g., potassium) (3-5, 7, 8, 10-12). The optimal long-term storage condition for most biological specimens, and in particular blood components, is in an ultrafreezer (–70°C) or in vapor-phase liquid nitrogen (–140°C to –196°C), because of reduced protease and RNase activity and excellent thermostability at these temperatures (2, 3-5, 8, 13). While freezing specimens is ideal for long term storage, often it is not possible in epidemiological studies to process, aliquot and freeze specimens immediately, and specimens are stored either at room temperature or refrigerated temperatures prior to aliquoting and freezing. In some situations, these delays may affect analytes (2-5, 7). A recent pilot study for the UK Biobank evaluated 30 hematological and clinical chemistry analytes for changes during short-term refrigerated storage for up to 36 hours, with relatively small decreases in concentration over time were observed for most analytes (1–3% per 12 hours) (14).

NCS procedures were developed to minimize variability in specimen handling through standardized central processing, aliquoting, and short- and long-term storage of biospecimens. The ongoing Vanguard Pilot phase of the NCS is intended to evaluate the feasibility, acceptability, and cost of recruitment strategies, as well as study procedures and outcome assessments under consideration for use in the NCS (1). Standard operating procedures for handling blood biospecimens involve the following sequence of events: i) sample collection at local sites; ii) centrifugation within two hours; iii) overnight shipment of unopened primary collection containers at 2–8°C to a central repository within 24 hours;

and iv) aliquoting upon receipt at the central repository, followed by long-term vapor-phase liquid nitrogen storage. Modifications to these general procedures are implemented as appropriate, depending on the specimen type (e.g., serum, plasma, or whole blood) and type of blood tube (e.g., acid citrate dextrose tubes used for lymphocyte cryopreservation).

After literature review and expert consultation, we identified several analytes from different biochemical categories for which it was unclear to what extent deviations from NCS specified preanalytical conditions and handling could influence measurement results. Consequently, a pilot study was performed for eight different analytes to examine the effects of planned variation in preanalytical conditions: delays in centrifugation, delays in freezing, delays in serum or plasma separation from cells, delays in preservative addition, and impact of different blood tube types.

Materials and Methods

STUDY POPULATION

Eligible subjects were premenopausal women at least 18 years of age. Participants were excluded if they had hemophilia or other bleeding disorder, were on anticoagulant therapy, or had undergone cancer chemotherapy within the past four weeks. Written informed consent was obtained from all participants. No personal identifying information was retained with the specimens. The study protocol was reviewed and approved by an Office of Human Research Protections (OHRP) registered Institutional Review Board.

BIOSPECIMEN COLLECTION AND PROCESSING

Non-fasting peripheral venous blood was collected and processed at Westat (Rockville, MD). The maximum blood volume collected per participant was 80 mL. Blood was collected from the antecubital vein using a 21-gauge butterfly needle into preselected Vacutainer™ (Becton Dickinson [BD]; Franklin Lakes, NJ) blood collection tubes from single, unexpired lots. Blood collection tubes were 8.5 mL draw volume serum separator tubes (SST; BD367988), 5 mL plasma preparation tubes (PPT; BD362788) containing K₂EDTA, 8.5 mL LBD™ P100 tubes containing K₂EDTA and protein stabilizers (P100; BD366455), 5 mL no-additive serum (red top; BD366441) tubes, and 5 mL K₂EDTA plasma (lavender top; BD362788) tubes, depending on the proposed laboratory measure (Table 1). Blood tubes and times for specimen processing, storing, and aliquoting were based on planned NCS procedures and selected to evaluate possible deviations therefrom.

Specimens were collected from 151 women for the assessment of multiple analytes and handling conditions. Blood specimens were collected over a seven-week period beginning in February 2009. Measurements for each combination of analyte and handling condition were performed on 20 unique study participants (Table 1). Blood from the same participant and blood from the same tubes was used to study more than one combination (Supplemental Table 1 and Supplemental Figure 1). For example, the effects of delays in centrifugation and freezing delay on measurement of interleukin-6 (IL-6) were assessed using blood from the same 28 participants; similarly, aliquots from the same collection tube were used for

measurement of % CDT, insulin, and IGF-1 to assess effects of delays in centrifugation and freezing delay.

Multiple tubes were collected from each participant and processed according to detailed procedures specific for each combination of analyte and handling condition (Supplemental Figures 1-4). Hard copy data collection forms and the Westat Biospecimen and Environmental Sample Tracking (BEST®) system were used to record and monitor specimen collection and processing events at defined time intervals. Specimen collection forms and data logs were reviewed twice daily. In a retrospective quality assurance review of actual versus nominal processing times for those analytes and conditions where statistically significant differences were observed, median relative bias was 4.3% (data not shown).

Centrifugation was unrefrigerated. P100 tubes were spun for 30 minutes at 6000 rpm ($1228 \times g$) in an ICE Microspin (International Equipment Co., Needham Hts., MA). All other tubes were centrifuged in a Hettich EBA20 Centrifuge (Buckinghamshire, England) for 15 minutes at 3500 rpm ($1000\text{--}1200 \times g$), in accordance with manufacturer instructions. Following sample processing, blood sample fractions (serum or plasma) were aliquoted into polypropylene cryovials in volumes sufficient to perform each laboratory assay in duplicate for each condition evaluated. Aliquots were stored at -70°C until transfer to the NCS repository (Fisher BioServices; Rockville, MD) approximately two months following completion of collection. Sample aliquots were stored at the repository in vapor-phase liquid nitrogen until laboratory analysis. Repository samples were shipped on dry ice to the analysis laboratory 18-20 months following initial collection.

Effects of Delays in Centrifugation of P100, PPT, and SST tubes—Four blood tubes per participant were obtained to assess post-collection delays in blood tube (P100, PPT, or SST) centrifugation of 0.5, 1.5, 3.5, and 5.5 hours (Supplemental Figure 1). The effects of centrifugation delays were evaluated for adrenocorticotrophic hormone (ACTH), interleukin-6 (IL-6), thyroxine (free T_4), percent carbohydrate deficient transferrin (% CDT), insulin, and insulin-like growth factor 1 (IGF-1). Tubes were stored at ambient temperature before centrifugation. After centrifugation, serum or plasma was aliquoted and stored at -70°C .

Effects of Delays in Freezing of P100, PPT, and SST tubes—Four blood tubes per participant (P100, PPT, or SST) were used to assess post-centrifugation freezing delays for ACTH, IL-6, free T_4 , % CDT, insulin, and IGF-1 (Supplemental Figure 2). Tubes were stored at ambient temperature before centrifugation. Each blood tube was centrifuged at two hours post-collection. Immediately after centrifugation, serum or plasma from the first tube from each participant was aliquoted and stored at -70°C . The remaining tubes from each set were stored at $2\text{--}8^{\circ}\text{C}$ for 24, 48, or 72 hours until aliquoting and storage at -70°C .

Delay in Serum Separation from Cells for Specimens Collected in Red-Top Tubes—Four no-additive serum (Red-Top) tubes were collected per participant to examine delays in serum separation from cells for estradiol measurement (Supplemental Figure 3). Tubes were held at $2\text{--}8^{\circ}\text{C}$ and centrifuged two hours after collection. Serum was removed

from the first tube, aliquoted, and frozen at -70°C . The next three tubes were stored at 2-8 $^{\circ}\text{C}$ for 24, 48, or 72 hours. At each time point, one tube was re-centrifuged and serum aliquoted and frozen at -70°C .

Separation and Additive Delay—To test delays in addition of metaphosphoric acid (MPA) preservative to serum specimens for measurement of vitamin C, serum was collected and processed according to procedures described above for “Delay in Serum Separation from Cells for Specimens Collected in Red Top Tubes.” At 0, 24, 48, or 72 hours, serum was aliquoted into cryovials containing 1mL of 2% MPA and frozen at -70°C (Supplemental Figure 3).

Tube Type—For each participant, one P100 and two K_2EDTA anticoagulated whole blood tubes were collected to evaluate the effect of tube type on ACTH measurement (Supplemental Figure 4). Aprotinin, a serine protease inhibitor, was added to one lavender top tube. The second lavender top tube contained no additional inhibitor. Blood tubes remained at room temperature and were centrifuged at two hours after collection. After centrifugation, plasma was aliquoted and frozen at -70°C .

LABORATORY ASSAYS

De-identified, coded frozen specimen aliquots were shipped on dry ice for laboratory analysis to Quest Diagnostics Nichols Institute (San Juan Capistrano, CA) or to the Division of Laboratory Sciences, National Center for Environmental Health (NCEH), Centers for Disease Control and Prevention (CDC), and maintained at -70°C until testing. Pooled quality control (QC) materials (serum and plasma, ProMedDx [Norton, MA]) were aliquoted to create blinded replicate QC samples which were seeded at a rate of 5% by the NCS repository into each batch of experimental samples prior to shipment for analysis. Two replicate aliquots of each experimental sample were analyzed for each experimental time point. Laboratory personnel were blinded to specimen identity. Specimens were shipped to laboratories in a single batch. Analytes were measured within two to four weeks after specimen shipment. All frozen samples were thawed at ambient temperature prior to measurement.

Percent CDT (nephelometry, Siemens BNII), insulin (immunoassay, Siemens Immulite), IGF-1 (LC/MS/MS), free T_4 (direct equilibrium dialysis, radioimmunoassay; Siemens Advia Centaur), ACTH (immunoassay; Siemens Immulite); estradiol (LC/MS/MS), and IL-6 (highly sensitive ELISA; R&D Systems Quantikine HS Human IL-6 Immunoassay) assays were performed according to standard procedures at Quest Diagnostics Nichols Institute. Vitamin C measurements were performed at the CDC with a laboratory developed test using isocratic high performance liquid chromatography (HPLC) with electrochemical detection at 650 mV using the Agilent High Performance Liquid Chromatography system (Agilent Corporation Wilmington, DE) (15). For each participant, all samples for a given assay were analyzed in a single run to avoid confusing within-person differences with differences caused by assay variation.

STATISTICAL METHODS

Each experiment tested a set of alternate sample processing procedures (e.g., delay in centrifugation) on measurements of a specified analyte (e.g., ACTH concentration). Assay results were log-transformed for statistical analysis. The different components of variance of the prediction errors associated with concentration differences between subjects, between blood tubes from the same subject, and between aliquots from the same blood tube (replicates) were assumed to be normally distributed and additive in the transformed scale. To avoid introduction of bias in estimating the characteristics of the distribution of measurement values, with consequent distortion of regression coefficients and their standard errors and reduced power in hypothesis tests, we replaced measurement values below the limit of detection (LOD) with a value calculated as the LOD divided by the square root of 2 (16, 17). Sample sizes for each analyte and handling condition were designed to permit detection of critical mean differences in analyte concentration of at least 10% between baseline and at least one other time point, with $\alpha=0.01$ and 80 percent power. All p -values were two sided.

Data were analyzed using mixed linear models, using the SAS MIXED procedure (SAS v. 9.2; Cary, NC). F-test p -values for differences among handling condition categories were used to assess statistical significance, using a critical value of 0.05. Models with alternate transformations and variance assumptions were examined, giving similar results and identical conclusions regarding statistically significant differences. Predicted least-square means in the log-transformed scale are presented as geometric means in the measurement units and referred to as predicted geometric means. Confidence intervals for comparing geometric means among handling conditions are least square means, calculated by treating the subjects as fixed effects, transformed to the measurement scale.

Estimates of variance (V) for each variance component when predicting the log-transformed measurements were obtained from the mixed model and are presented as coefficients of variation (CVs) in the measurement units, using the formula: $CV = \sqrt{e^V - 1}$. The CVs describe variation among a set of subjects (between-subject CV), set of blood tubes from the same subjects (between-tube CV), and sets of replicate measurements from the same blood tubes (measurement CV). Assay precision was also estimated using from QC samples that were analyzed with the test samples (pooled CV from QC samples; Supplemental Table 2).

Results

A total of 2,825 specimen aliquots were assayed. Overall, only 0.6% of specimens had analyte concentrations below the limit of detection for a specific assay and condition (range: 0-8.5%, Supplemental Table 2). The greatest percentage of specimens with concentrations below the detection limit (8.5%) was observed for the experiment examining the impact of centrifugation delays on ACTH measurement. Pooled estimates for CVs across QC specimens for all assays and experimental conditions were 20%, except for ACTH (32%) and estradiol (43%). For replicate measurements from the same blood tubes, or measurement CV, all CVs were 17% except estradiol (65%). Average values and

confidence intervals for all measured analytes were within reported reference ranges (data not shown) (18-19).

The effect of centrifugation delay for specimens collected in P100, PPT, and SST tubes was examined for ACTH, IL-6, free T₄, % CDT, insulin, and IGF-1. The observed concentrations of IL-6 and insulin declined in response to centrifugation delay ($P < 0.0001$; Table 2 and Supplemental Figure 5). No statistically significant changes were observed for concentrations of other analytes in response to centrifugation delay.

Effects of delays in freezing of 24, 48, or 72 hours following centrifugation were examined for ACTH, IL-6, free T₄, % CDT, insulin, and IGF-1. A trend of increased measured concentration of ACTH was observed ($P = 0.0014$; Table 3 and Supplemental Figure 5). No statistically significant differences were observed for other analytes.

Several other conditions were examined, including delay in separation of serum from cells for measurement of estradiol, delay in serum separation and MPA preservative addition for vitamin C measurement, and different blood tube types for ACTH measurement (P100 tube and K₂EDTA plasma tube with or without addition of protease inhibitor). None of these conditions resulted in a statistically significant change in the concentration of the analyte tested (Table 4). Analytical measurement variation for estradiol was relatively high (Supplemental Table 2), which may limit ability to detect potential differences among various preanalytical experimental conditions.

Discussion

The purpose of this study was to examine the effects of preanalytical sample handling conditions that may be encountered during NCS biospecimen procedures for several proposed NCS measures. Overall, most analytes evaluated were stable under the test conditions explored, with no statistically significant changes in concentration observed. However, we found that levels of plasma IL-6 and serum insulin decreased with delays in time to initial centrifugation, and ACTH concentrations increased after a delay in sample freezing.

We observed a decline in IL-6 concentration in response to delays in centrifugation (i.e., during prolonged exposure of plasma to blood cellular components). Previous studies have also observed significant IL-6 declines in EDTA plasma in response to processing delays, using native biospecimens after four hours (20) and spiked biospecimens measured 0.25 to 24 hours post-collection (21). Longer post-collection processing delays (24, 48, or 72 hours) significantly affected IL-6 values in trauma patients but not in healthy controls (22). The observed decline in IL-6 in our samples could be the result of clearance by the cellular component of the specimens (20). After centrifugation and separation of plasma from cellular components in blood, concentrations of IL-6 appeared stable during storage at 4°C. This finding is consistent with the observation that IL-6 is stable during repeated free-thaw cycles (20-21). Taken together, these results suggest that IL-6 is degraded in unprocessed biospecimens but can be a stable cytokine for measurement in epidemiologic studies if separation of plasma from cellular blood components is not delayed.

Plasma insulin concentrations decreased significantly with a delay in centrifugation after blood collection. Most of the change was observed after a 1.5 hour delay, but declines continued throughout the 5.5 hours studied. Decreases of this magnitude (~22%) are unlikely to have consequences for the diagnostic utility of insulin measurement, which is used only occasionally for diabetes management and is typically limited to identifying patients with severe insulin resistance or insulinomas (23). Nevertheless, insulin measurements continue to be widely used in population-based studies to assess insulin sensitivity by the homeostasis model assessment-estimated insulin resistance (HOMA-IR), which allows for a simple estimate of insulin sensitivity and beta-cell function from fasting plasma insulin and glucose concentrations (24). HOMA-IR estimates have also been utilized in longitudinal studies to assess the effects of various interventions on insulin resistance (25-27). For both cross-sectional (28) and longitudinal studies, it clearly is important to minimize potential preanalytic error and variability associated with sample preparation procedures, since a delay in processing would yield a lower insulin value and hence inappropriately suggest greater observed insulin sensitivity compared with a serum sample processed immediately after blood clotting.

Plasma ACTH levels in specimens collected in protease inhibitor-containing BD-P100 tubes were unaffected by delays in centrifugation. Previous studies have shown that ACTH levels can decrease with time in whole blood and plasma when collected in tubes using EDTA as the anticoagulant (29-30). Thus, tubes with protease inhibitor, such as contained in BD-P100 tubes, appear to provide protection against analyte loss during collection and processing, consistent with a previous study of aprotinin (30). However, it is notable that when different tube types were compared, with and without additional protease inhibitor, no difference was observed in ACTH concentration when specimens were processed two hours after collection. Storage at 4°C resulted in a continuous increase in plasma ACTH levels as measured at time points up to 72 hours (approximately 6%/24 hour). The increase at 72 hours is within the measured analytic variability of the assay (Supplemental Table 1), so such changes are unlikely to have diagnostic consequences. The mechanism responsible for the increase in ACTH levels during short-term refrigerated storage (freezing delay) is uncertain. One possibility is that ACTH precursors (e.g., pro-ACTH and pro-opiomelanocortin [POMC]) that are present in blood at concentrations approximately 5-fold higher than ACTH (31) with time may express altered immunoreactivity and cause an apparent increase in ACTH concentrations. To our knowledge, the stability of such precursors has not been reported in the literature. Further studies are needed to confirm this concept.

Vitamin C is known to be unstable in serum and plasma. Nevertheless, we observed no effect of delaying MPA addition to stabilize ascorbic acid with specimens held at refrigerated temperatures up to three days. This finding extends previous work showing that vitamin C is stable in whole blood held at 4°C for one day (32-35).

The strengths of this study include the systematic approach taken to minimize instability of each analyte. For example, we expected that ACTH, because of its structure, might benefit from exogenous protease inhibition. To develop practical information, planned variations were selected to provide convenient end-points should unexpected delays in processing

arise, allowing information from this study to be used to make informed decisions about data reliability. An additional strength is that the subjects were relatively normal with respect to the means and ranges of values studied (data not shown).

There are several limitations of this study. One is the relatively small number of subjects included in the protocols. Although power calculations using the expected sources of variation predicted our ability to assess the null hypothesis, it remains possible that individual specimens may react differently than those in this study. For example, specimens from less well-nourished subjects or smokers might be less stable than those included in this study; lack of adequate antioxidant protection from foods or supplements, or destruction of antioxidants by tobacco smoke, might destabilize vitamin C. Because no epidemiological data were collected, it is not possible within this study to evaluate these factors. Another limitation is that the observed estradiol CV exceeded the value assumed in sample size calculation, which may have limited our ability to detect significant differences.

Conclusions

Determining the stability of proposed analytes in response to preanalytical conditions and handling helps to ensure high-quality specimens for study. Results from the methodologic studies reported here inform and enhance development of procedures for the NCS, plans for measurement of analytes in NCS biospecimens, and interpretation of results. Studies like this provide valuable information to inform the planning and conduct of epidemiologic studies. The importance of understanding the contribution of preanalytical conditions to clinical laboratory measurements is widely acknowledged but inadequately studied. Our observation that centrifugation delay significantly influences resulting insulin levels may have important implications for research investigations into glucose metabolism, metabolic syndrome, obesity, and related issues. Systematic evaluation of the impact of variation in preanalytical conditions on laboratory measurements can advance our understanding of biospecimen and clinical laboratory science and permit that knowledge to inform and improve the development, design, and quality of biomedical research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention, National Children's Study, National Institutes of Health, or U.S. Department of Health and Human Services.

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Abbreviations

ACTH	adrenocorticotrophic hormone
CDT	carbohydrate-deficient transferrin
free T₄	free thyroxine
IGF-1	insulin-like growth factor 1
IL-6	interleukin-6
MPA	metaphosphoric acid
NCS	National Children's Study

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Table 1

Summary of Analytes and Conditions for Stability Testing of Biospecimens

Analyte	Matrix (Blood Collection Tube)	Conditions				
		Delays in Centrifugation	Short Term Refrigerated Storage	Delay in Separation from Cells	Separation and Additive Delay	Tube Type
		Number of Measurements ^a (Number of Participants ^b)				
ACTH	Plasma (P100)	164 (21)	160 (20)			
IL-6	Plasma (PPT)	238 (30)	232 (29)			
Free T ₄ (Thyroxine)	Serum (SST)	165 (21)	160 (20)			
% CDT	Serum (SST)	158	(21)	148	(20)	
Insulin		164		159		
IGF-1		165		160		
Estradiol	Serum (Red Top Tube)			160 (20)		
Vitamin C					160 (20)	
ACTH	Plasma (P100; Lavender; Lavender plus inhibitor)					432 (37) ^c

^a A small number of aliquots were missing laboratory results due to insufficient volume, hemolyzed specimens, or laboratory determination of inconsistent results.

^b Number of unique participants per specified analyte and condition. It should be noted that study participants were included in multiple analyte-handling conditions (described in Supplemental Table 1). The total number of unique participants in the study was 151.

^c Data from one subject was excluded from the analysis because all values for that subject were below the detection limit.

Table 2

Effects of Delays in Centrifugation on Analyte Concentrations in Biospecimens

Analyte	Concentration ^a (95% Confidence Interval)				P-Value
	0.5 hours	1.5 hours	3.5 hours	5.5 hours	
ACTH (pg/mL)	13.4 (12.8-14.1)	13.5 (12.8-14.1)	13.4 (12.8-14.1)	13.2 (12.6-13.9)	0.97
IL-6 (pg/mL)	1.29 (1.24-1.34)	1.23 (1.19-1.28)	1.06 (1.02-1.10)	0.99 (0.95-1.03)	<0.0001
Free T ₄ (ng/dL)	1.41 (1.37-1.46)	1.36 (1.32-1.41)	1.43 (1.39-1.48)	1.41 (1.37-1.46)	0.20
% CDT	1.83 (1.76-1.90)	1.86 (1.79-1.93)	1.84 (1.77-1.91)	1.84 (1.77-1.91)	0.98
Insulin (μ IU/mL)	14.8 (14.0-15.5)	12.7 (12.1-13.4)	11.9 (11.3-12.5)	11.5 (10.9-12.1)	<0.0001
IGF-1 (ng/mL)	138.6 (133.4-144.0)	138.9 (133.7-144.2)	136.8 (131.7-142.0)	146.3 (140.7-152.1)	0.13

^aConcentrations are predicted geometric means for each time point and analyte.

SI unit conversion factors: ACTH, pg/mL \times 0.22=pmol/L; IL-6, pg/mL \times 0.131=IU/mL; free T₄, ng/dL \times 12.871=pmol/L; % CDT, % \times 1=%; insulin, μ IU/mL \times 6.00=pmol/L; IGF-1, ng/mL \times 0.131=nmol/L.

Table 3

Effects of Short Term Refrigerated Storage on Analyte Concentrations in Biospecimens

Analyte	Concentration ^a (95% Confidence Interval)				P-Value
	0 hours	24 hours	48 hours	72 hours	
ACTH (pg/mL)	12.4 (11.7-13.1)	13.2 (12.5-14.0)	14.3 (13.5-15.1)	14.8 (14.0-15.6)	0.0014
IL-6 (pg/mL)	1.15 (1.10-1.20)	1.12 (1.08-1.17)	1.10 (1.05-1.15)	1.10 (1.05-1.15)	0.51
Free T ₄ (ng/dL)	1.44 (1.40-1.49)	1.41 (1.37-1.46)	1.47 (1.42-1.52)	1.43 (1.39-1.48)	0.43
% CDT	1.83 (1.75-1.91)	1.85 (1.78-1.93)	1.78 (1.71-1.85)	1.80 (1.73-1.88)	0.65
Insulin (μ IU/mL)	15.2 (14.3-16.1)	14.7 (13.9-15.6)	14.7 (13.9-15.6)	13.7 (12.9-14.6)	0.22
IGF-1 (ng/mL)	143.1 (138.0-148.3)	140.1 (135.2-145.2)	140.3 (135.3-145.4)	140.2 (135.3-145.4)	0.84

^aConcentrations are predicted geometric means for each time point and analyte.

SI unit conversion factors: ACTH, pg/mL \times 0.22=pmol/L; IL-6, pg/mL \times 0.131=IU/mL; free T₄, ng/dL \times 12.871=pmol/L; % CDT, % \times 1=%; insulin, μ IU/mL \times 6.00=pmol/L; IGF-1, ng/mL \times 0.131=nmol/L.

Table 4

Effects of Delays in Separation from Cells, Additive Delay, and Tube Type on Analyte Concentrations in Biospecimens

Analyte	Concentration ^a (95% Confidence Interval)				P-Value
	0 hours	24 hours	48 hours	72 hours	
	<i>Delay in Separation from Cells</i>				
Estradiol (pg/mL)	30.5 (25.6-36.4)	34.0 (28.5-40.5)	37.4 (31.4-44.6)	31.2 (26.1-37.1)	0.41
	<i>Separation and Additive Delay (MPA) ^b</i>				
Vitamin C (mg/dL)	1.12 (1.11-1.14)	1.14 (1.12-1.15)	1.12 (1.10-1.13)	1.12 (1.11-1.14)	0.42
	<i>Tube Type</i>				
	Lavender tube plus inhibitor	Lavender tube	P100 tube		
ACTH (pg/mL)	14.8 (14.5-15.0)	14.9 (14.6-15.2)	14.8 (14.5-15.1)		0.78

^a Concentrations are predicted geometric means for each time point and analyte.

^b Separation (removal from blood tube) and additive (MPA) delay prior to aliquoting and freezing the specimen was performed as described in "Materials and Methods".

SI conversion factors: Estradiol, pg/mL×3.671=pmol/L; Vitamin C, mg/dL×56.78=μmol/L; ACTH, pg/mL×0.22=pmol/L.