

AD _____

GRANT NUMBER: DAMD17-94-J-4265

TITLE: Active Follow-up of Participants of a Population-Based Specimen Bank (Plasma, DNA, RBC)

PRINCIPAL INVESTIGATOR: Dr. Kathy J. Helzlsouer

CONTRACTING ORGANIZATION: Johns Hopkins University
School of Hygiene and Public Health
Baltimore, Maryland 21205

REPORT DATE: October 1995

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

19960124 016

DTIC QUALITY INSPECTED 1

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE October 1995	3. REPORT TYPE AND DATES COVERED Annual 1 Oct 94 - 30 Sep 95	
4. TITLE AND SUBTITLE Active Follow-up of Participants of a Population-Based Specimen Bank (Plasma, DNA, RBC)		5. FUNDING NUMBERS DAMD17-94-J-4265	
6. AUTHOR(S) Dr. Kathy J. Helzlsouer			
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Johns Hopkins University School of Hygiene and Public Health Baltimore, Maryland 21205		8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012		10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES			
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited		12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) The objectives of this proposal are to expand the resources of our population-based specimen bank (CLUE II) by collecting additional information on family history and breast cancer risk factors and to continue plasma stability studies. From May through November 1989, 32,320 blood specimens were collected from residents of Washington County, Maryland and the surrounding tri-state area. Participants donated 20 ml of blood, gave a brief medical history, completed a food frequency questionnaire and provided a nail sample. An active follow-up of the cohort will be instituted to collect and update information on breast cancer risk factors, including an extensive family history. Stability studies of Micronutrients and hormones in years 5 and 9 from the time of initial blood collection will be conducted.			
14. SUBJECT TERMS Cohort, specimen bank, breast cancer			15. NUMBER 13
			16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited

GENERAL INSTRUCTIONS FOR COMPLETING SF 298

The Report Documentation Page (RDP) is used in announcing and cataloging reports. It is important that this information be consistent with the rest of the report, particularly the cover and title page. Instructions for filling in each block of the form follow. It is important to *stay within the lines* to meet *optical scanning requirements*.

Block 1. Agency Use Only (Leave blank).

Block 2. Report Date. Full publication date including day, month, and year, if available (e.g. 1 Jan 88). Must cite at least the year.

Block 3. Type of Report and Dates Covered. State whether report is interim, final, etc. If applicable, enter inclusive report dates (e.g. 10 Jun 87 - 30 Jun 88).

Block 4. Title and Subtitle. A title is taken from the part of the report that provides the most meaningful and complete information. When a report is prepared in more than one volume, repeat the primary title, add volume number, and include subtitle for the specific volume. On classified documents enter the title classification in parentheses.

Block 5. Funding Numbers. To include contract and grant numbers; may include program element number(s), project number(s), task number(s), and work unit number(s). Use the following labels:

C - Contract	PR - Project
G - Grant	TA - Task
PE - Program Element	WU - Work Unit Accession No.

Block 6. Author(s). Name(s) of person(s) responsible for writing the report, performing the research, or credited with the content of the report. If editor or compiler, this should follow the name(s).

Block 7. Performing Organization Name(s) and Address(es). Self-explanatory.

Block 8. Performing Organization Report Number. Enter the unique alphanumeric report number(s) assigned by the organization performing the report.

Block 9. Sponsoring/Monitoring Agency Name(s) and Address(es). Self-explanatory.

Block 10. Sponsoring/Monitoring Agency Report Number. (If known)

Block 11. Supplementary Notes. Enter information not included elsewhere such as: Prepared in cooperation with...; Trans. of...; To be published in.... When a report is revised, include a statement whether the new report supersedes or supplements the older report.

Block 12a. Distribution/Availability Statement. Denotes public availability or limitations. Cite any availability to the public. Enter additional limitations or special markings in all capitals (e.g. NOFORN, REL, ITAR).

DOD - See DoDD 5230.24, "Distribution Statements on Technical Documents."

DOE - See authorities.

NASA - See Handbook NHB 2200.2.

NTIS - Leave blank.

Block 12b. Distribution Code.

DOD - Leave blank.

DOE - Enter DOE distribution categories from the Standard Distribution for Unclassified Scientific and Technical Reports.

NASA - Leave blank.

NTIS - Leave blank.

Block 13. Abstract. Include a brief (*Maximum 200 words*) factual summary of the most significant information contained in the report.

Block 14. Subject Terms. Keywords or phrases identifying major subjects in the report.

Block 15. Number of Pages. Enter the total number of pages.

Block 16. Price Code. Enter appropriate price code (*NTIS only*).

Blocks 17. - 19. Security Classifications. Self-explanatory. Enter U.S. Security Classification in accordance with U.S. Security Regulations (i.e., UNCLASSIFIED). If form contains classified information, stamp classification on the top and bottom of the page.

Block 20. Limitation of Abstract. This block must be completed to assign a limitation to the abstract. Enter either UL (unlimited) or SAR (same as report). An entry in this block is necessary if the abstract is to be limited. If blank, the abstract is assumed to be unlimited.

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

 Where copyrighted material is quoted, permission has been obtained to use such material.

 Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

 Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

 In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

 For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

 In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

 In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

 In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.



PI - Signature 10/24/95
Date

TABLE OF CONTENTS

Front Cover	1
SF 298 Report Documentation Page	2
Foreword	3
Table of Contents	4
Introduction	5-11
Body: Progress Report Year 1	11-12
Conclusions	12
References	13

INTRODUCTION

Objective:

The objective of this proposal is to expand the resources of our existing population-based specimen banks (plasma, white blood cells, red blood cells and nail samples) by collecting additional information on family history and breast cancer risk factors so that gene-environment interactions leading to breast cancer may be investigated.

Background of Previous Work:

The CLUE Specimen Banks

From August through November, 1974, a total of 25,620 serum specimens were collected in Washington County, MD for a research serum bank (CLUE I). An additional 182 specimens were obtained in the summer of 1975, for a total of 25,802. Linkage of the records from this program to those of a private census in the summer of 1975 indicated that almost a third of the adult population of the county had participated. Participation was best in the age group 35 to 65 years, and was slightly better among females, the better-educated, and nonsmokers.

A second program was conducted from May through November, 1989 collecting 32,320 blood specimens (CLUE II). Of these specimens, 8118 were collected from individuals who had also participated in CLUE I. Participants donated 20 ml of blood, gave a brief medical history, completed a food frequency questionnaire and returned a toenail clipping for trace metal studies. Somewhat greater participation was obtained among older persons, possibly because a free cholesterol test was offered as an incentive to participate. Quality checks showed good agreement between cholesterol determinations by the local medical laboratory and the CDC-approved laboratory at Johns Hopkins Hospital.

In addition to storing two 5 ml aliquot of plasma, another 0.7 ml aliquot was preserved with 0.7 ml of 10% metaphosphoric acid to allow subsequent ascorbic acid assays. The buffy coat, providing DNA, and RBC fractions were also stored. The two aliquots of plasma from each person are stored in separate freezers. All specimens have been kept at -70° C or colder.

CLUE I has been extensively used to examine the potential protective effect of specific micronutrients against the development of cancer including breast cancer (1). The role of endogenous hormones in the development of breast cancer has also been examined using the resources of this serum bank (2). With the maturation of the cohort from CLUE II, the availability of DNA in addition to plasma, and the technological advances of molecular biology we will be able to investigate etiologic, protective and

susceptibility factors leading to the development of breast cancer. In order to use the specimen bank to its fullest potential we require additional data on participants in the CLUE II cohort such as extended family history and other risk factors. Breast cancer risk factors such as family history of the disease, age at pregnancy and parity change over time requiring institution of active follow-up of the cohort. These risk factors should be taken into account in investigations of serologic precursors or susceptibility factors associated with breast cancer. The ability to identify and investigate families with multiple members affected by breast cancer is a valuable resource for studying the role and contribution of inherited susceptibility factors to the development of breast cancer. Since these inherited factors may be passed through the mother and father it is essential to obtain information on the family history of cancer of male and female participants in the cohort.

Stability studies of micronutrients and hormones have been conducted up to 42 months. We propose to continue these studies up to 15 years. The resources of serum and specimen banks become more valuable with time as the number of cancer cases developing among members increases with aging of the cohort. It is essential to assess the stability over many years of factors being studied in order to optimally utilize these valuable resources. A literature review of the effects of long-term freezer storage on concentrations of retinol, beta-carotene, and alpha-tocopherol in repeated assays of the same serum or plasma pools shows that most of the studies are deficient because of very small numbers of observations, imprecise descriptions of procedures, and/or short periods of storage (3). The literature is even more scanty with respect to long-term storage effects on concentrations of other carotenoids, ascorbic acid, and hormones.

We have been interested in storage effects for a long time, and have published findings from our nested case-control studies and those of others (3). There was suggestive evidence of considerable loss of alpha-feto-protein after 5 years of storage (4). Retinol appears to be stable for at least 15 years at temperatures as warm as -20°C , alpha-tocopherol at temperatures as warm as -40°C , and beta-carotene at temperatures of -70°C or lower (3). A recent study using serum specimens collected in 1974 and assayed in 1991 showed sufficient micronutrient levels to demonstrate important case-control differences (5). However, more convincing storage studies are those that assay aliquots of the same specimens at different time periods. Such information is sparse in the literature and is limited almost entirely to retinol (3). To obtain information based on periodic assays of the same plasma specimen stored at -70°C , we initiated a study in 1990 using 40 pools of plasma for micronutrients and 18 pools for hormones. Assays have been done at 1 year, 2 years, and 3.5 years after starting storage. Analytes include ascorbic acid (preserved with metaphosphoric acid), retinol, total carotenoids, alpha- and beta-carotene, cryptoxanthin, lutein, lycopene, estrone, estradiol, progesterone, testosterone, and sex-hormone-binding globulin. The effects of repeated freezing and thawing have also been studied, first on a pilot basis and more recently in a full-scale study. The pilot study showed no demonstrable effects of up to four cycles on retinol, total carotenoids, beta-carotene, prolactin, lutropin, and follitropin (6). The full scale study looked at the effects of up to 10 cycles.

Analysis is under way, with preliminary results indicating that there are no demonstrable differences, at least up to six freeze-thaw cycles. Analyses of the duration of storage study have also started. There appear to be only trivial changes in ascorbic acid concentrations over the 3.5 year period. Inspection of the results for the other micronutrients reveals no gross changes. There appears to be considerable variability in the assays for estrone, estradiol, progesterone, testosterone and sex-hormone-binding globulin but no evidence of appreciable losses so far.

Although differences between cases and controls can still be informative as long as reasonable concentrations of analytes persist, it is important to know the degree of loss with storage time both for planning and for interpreting studies based on stored serum, plasma or blood cells. From the practical point of view it is almost essential to have this information to satisfy study sections and reviewers of manuscripts. In our experience over the past 20 years, such queries have been almost universal.

Cancer Register:

A cancer register for Washington County has been maintained since 1958, with records dating back to 1948. Its primary source is discharge records from the Washington County Hospital, the only general hospital in the county. Because of its well-equipped and staffed Oncology Service, the hospital tends to draw patients from surrounding counties rather than to lose them to other institutions. Cases are also ascertained from death certificates of Washington County residents which are under the custody of our unit acting as a branch of the Health Department. Comparisons of observed cases in the populations that donated blood for the serum bank with the number expected on the basis of race-sex-age specific rates from the SEER registries suggest that reporting is essentially complete for this subpopulation. The only major deficit is for stomach cancer; the only major excess is for prostate cancer. Records of reported county cancer cases are computer-linked to the lists of serum bank donors. Matching on variables such as age, date of blood donation, day of menstrual cycle are readily accomplished. Age matching is often possible within a few days or weeks.

Purpose of Present Work

Further progress in understanding the etiology of breast cancer and in developing new methods for early detection and prevention requires investigations to consider both genetic and environmental influences and their interaction in the development of breast cancer. The purpose of this proposal is to expand the resources of our existing population-based specimen bank by updating baseline information and obtaining information on breast cancer risk factors, particularly family history of cancer. Gene-environment interactions may then be explored to the fullest potential. The availability of family history information will permit the targeting of high risk individuals, based on familial factors, for investigations of possible inherited susceptibility factors.

The second major objective of this proposal is to continue to obtain fundamental information on changes in the concentration of various analytes in plasma and blood cells associated with long-term storage at -70°C. The plasma analytes are retinol, ascorbic acid, the major carotenoids found in human serum, alpha- and gamma-tocopherol, sex-hormone-binding globulin, and selected steroid hormones. The hypothesis to be tested in each case is the null hypothesis that there will be no change in concentration with storage time. The resources of banks become more valuable with time as cohorts mature providing more cases of cancer for investigation, and as new hypothesis and techniques become available for evaluation. Storage studies will provide basic information on the stability of markers of exposure, susceptibility and protection from breast cancer that is almost nonexistent. This information is essential for planning and interpreting studies using stored plasma, red blood cells and white blood cells (DNA).

Technical Objectives (Specific aims)

To enhance the resources of the existing population-based specimen bank we propose to:

1. Expand information collected at CLUE II in 1989. For example, total years of smoking and medication use, especially exogenous hormones.
2. Update information collected at CLUE II. For example, changes in marital status, smoking status, use of exogenous hormones, weight changes and dietary changes.
3. Obtain additional information relevant to breast cancer. For example, detailed cancer family history, number and timing of pregnancies, preventive health behavior (breast cancer screening), history of breast biopsies and occupational exposures.
4. Expand the cancer registry population base by including CLUE II participants who reside outside of Washington County.

To facilitate the appropriate use of the resources and to enhance interpretation of studies performed on the cohort we propose to:

5. Continue the study of the effects of long-term freezer storage of plasma at -70°C on its content of antioxidant nutrients, such as retinol (including retinoic acid and retinol palmitate), ascorbic acid, carotenoids, and tocopherols, as well as its content of hormones and lipoproteins.

Methods

Eligible Population

CLUE II participants living within a 30 mile radius of the intersection of Interstates 70 and 81 will be included in the active follow-up cohort. This geographic boundary includes all of Washington County and parts of surrounding counties, extending into Pennsylvania and West Virginia. This boundary includes 30,100 of the 32,320 CLUE II participants. The boundary was chosen to form a homogeneous cohort for the long-term follow-up. The CLUE II samples excluded from the cohort (2,220) will be used for our storage studies and cross-sectional investigations that can be conducted using data collected at baseline. To date, Over 340 cases of breast cancer have occurred among CLUE I and CLUE II participants.

Follow-up Procedure

CLUE II participants located in the geographic boundary of the long-term follow-up cohort will be mailed a questionnaire every 2 years. We will require considerable clerical work to update addresses from the time of CLUE II participation since all rural routes have been eliminated.

Non-respondents will be sent a second questionnaire. Telephone follow-up will be conducted for nonresponders after the second mailing. Telephone follow-up will also be carried out to clarify questionnaires completed by respondents. It is anticipated that 10 to 15% of family history questions will require further clarification by telephone follow-up. Resources available to search for current addresses of participant's whose questionnaires are returned due to incorrect mailing address include the Polk's City Directory for Hagerstown which covers Washington County and surrounding communities and the Hill-Donnelly Cross Reference Directory. Annual regional phone books (back to 1970) for Washington County and the Tri-state area are also available to trace address changes.

We expect to be able to trace at least 90% of CLUE II participants based on a previous experience with a telephone survey of menstrual histories of CLUE II women participants conducted in 1990. Several long-term cohort studies have been conducted in this community with consistently high participation rates.

As part of this study, we propose to collect additional data on CLUE II participants. Specifically, data will be collected from women on known risk factors for breast cancer. Examples of risk factors include: family history of cancer in first and second degree relatives, history of breast biopsy, type of benign breast disease, age at menarche, first birth, menopause, parity, months of lactation, use of exogenous hormones, height and weight and screening history. Updated risk factor information will be obtained at 2 year intervals. Updated food frequency information will be obtained at the second mailing. Because inherited susceptibility factors may be inherited through the mother and the father and because of recent evidence of common inherited and environmental factors for breast

and prostate cancer we will include male and female participants in the active follow-up cohort.

Based on data currently available we estimate the survival rate of participants to be approximately 95%. Information will be obtained from participants using a self-administered mailed questionnaire, with follow-up telephone interviews of non-respondents after a second mailing. Deceased CLUE participants will be identified by linking the CLUE II population to death certificates and by follow-up. The Training Center for Public Health Research is a repository for county death certificates. For deceased CLUE participants, next-of-kin will be sent a similar self-administered questionnaire. For people we cannot contact, the National Death Index will be accessed. Next-of-kin will be interviewed only on the initial round of follow-up.

Machine readable forms will be used for all questions except family history. Family history questionnaire does not lend itself to a machine readable format because name, age at diagnosis, and all causes of death must be recorded.

Methods for Study of Stability

The campaign for participation in CLUE II was conducted from May to November, 1989. Blood was drawn into a 20 ml Vacutainer containing heparin and was refrigerated at 4°C until it was delivered to the CLUE II laboratory within a few hours after being drawn. After centrifugation, 0.7 ml of plasma was added to 0.7 ml of 10% metaphosphoric acid. The remaining plasma was divided into two equal aliquots, the buffy coat was removed, as were 2 ml of red blood cells. Each of these specimens were placed in cryotubes and promptly frozen at -70°C.

For the stability studies of micronutrients and hormones, pools of plasma were created from the plasma of persons who lived outside of the study area and who had donated blood near the end of the campaign. For the micronutrients, 40 pools were created, each containing the plasma from four individuals. For hormone assays, 16 pools were created, each containing the plasma from eight individuals. The 40 micronutrient pools and the 16 hormone pools were each composed of equal numbers of pools from young and old men, and young and old women. A large reference pool was also created, so that four aliquots could be added to the micronutrient specimens and two to the hormone specimens.

Assays for micronutrients and hormones have been done at baseline, 12, 22, and 42 months after the median time of blood drawing. We propose to do three more rounds of assays. The assays will be done in the same laboratories at each round, using the same methods insofar as this is practical. If methods must be changed, assays will be done in duplicate by each method to determine the relationship between their results. Micronutrient assays have been done in the laboratory of Dr. Edward Norkus at Our Lady of Mercy Medical Center in New York City using high performance liquid chromatography. The micronutrients to be assayed include the retinoids, including retinyl palmitate, alpha- and

gamma-tocopherol, the carotenoids and ascorbic acid. The carotenoids include total carotenoids, alpha- and beta-carotenes, cryptoxanthin, lutein, and lycopene (7). Ascorbic acid will also be assayed by Dr. Norkus on plasma preserved with meta-phosphoric acid using high performance liquid chromatography (8). The hormone assays have been done in the laboratory of Dr. Christopher Longcope, University of Massachusetts. The hormones that have been assayed include estrone and estradiol, progesterone, androstenedione, testosterone and sex-hormone-binding globulin. Estrone, estradiol, testosterone, androstenedione, progesterone, and prolactin will be analyzed by radioimmunoassay (9). Inter- and intraassay coefficients of variation for these assays are approximately 10% and 7%, respectively. Sex hormone binding globulin capacity will be measured using the filter disc method of Mickelson and Petra (10). Inter- and intraassay coefficients of variation are reported to be 10.9% and 8.0% respectively (11).

The basic analysis of the data will be to calculate regression lines for each pool and to average the slopes for each age-sex group, and where appropriate for the total group. This approach assumes that an equal amount of analyte is lost in each unit of time. If there is evidence of appreciable change, this assumption will be checked by plotting amount of change and percentage change from baseline. If indicated, the data will be transformed before calculating the slopes of the regression lines.

BODY: PROGRESS REPORT YEAR 1

Task 1: Tracing of Participants

- a. The initial step undertaken was to computerize the database of participants to the 1989 Cohort (CLUE II). All names, addresses at study entry and unique study identification number of the 30,100 participants included in the 30 mile study region were entered.
- b. Name changes are being updated by searching published wedding announcements.
- c. We are actively tracing the participants and updating the mailing lists. This requires manual search of phone books, the Polk City Directory for Hagerstown, and the Hill-Donnelly Cross Reference Directory for Washington County to establish current addresses. To date 9600 of the participants have been traced. The tracing is very labor intensive and we have hired additional personnel to complete this task.
- d. Vital status is being determined by cross-referencing the participant list to death certificate data and searches of obituary notices.

Task 2: Questionnaire development:

Several meetings of the steering committee (Serologic Precursors Committee) were held throughout the year to develop the follow-up questionnaire. A draft questionnaire has been prepared. The final version of the questionnaire should be completed by November 6. Three meetings of the Steering Committee will be held from October 23 to November 6 to finalize the questionnaire.

The questionnaire will be printed in a machine-readable format. The steering committee has recommended that the first round of the questionnaire be devoted to the collection of new data and to include the Food Frequency Questionnaire, which was collected at baseline, in the second round of the questionnaire.

Task 3: Storage Effects

Aliquots for micronutrients have been submitted for year 6 assays. Aliquots have also been prepared for hormone assays. Analysis of rounds 1 and 2 of the storage effect revealed problems with reliability of the hormonal assay measurements. The hormone aliquots will be submitted for assays once this issue has been resolved.

CONCLUSIONS

Tracing of participants and updating of mailing lists are proceeding. Additional personnel have been hired to complete this task which is very labor intensive.

The questionnaire will be available for mailing to participants during the first quarter of Year 2.

Storage effects assays are in progress. Progress continues as planned. No major changes to the approved protocol have been necessary. Tasks for subsequent years will be completed as in the original protocol.

REFERENCES

1. Comstock GW, Bush TL, Helzlsouer K. Serum retinol, beta-carotene, vitamin E, and selenium as related to subsequent cancer of specific sites. *Am J Epidemiol* 1992;135:115-121.
2. Helzlsouer KJ, Alberg AJ, Bush TL, Longcope C, Gordon GB, Comstock GW. A Prospective Study of Endogenous hormones and breast cancer. *Cancer Detection and Prevention* 1994;18(2):79-85.
3. Comstock GW, Alberg AJ, Helzlsouer KJ. Effects of long-term freezer storage on concentrations of retinol, beta-carotene, and vitamin E in serum or plasma. *Clinical Chemistry* 1993;39:1075-8.
4. Helsing KJ, Comstock GW, Lau HL, Linkins S. Changes in alpha-fetoprotein concentrations during storage at -73°C. (Letter) *Clin Chem* 1994;30:1890.
5. Zheng W, Blot WJ, Diamond EL, Norkus EP, Spate V, Morris JS, Comstock GW. Serum micronutrient and the subsequent risk of oral and pharyngeal cancer. *Cancer Research* 1993;53:795-798.
6. Hsing AW, Comstock GW, Polk BF. Effects of repeated freezing and thawing on vitamins and hormones in serum. *Clin Chem* 1989;35:2145.
7. Craft NE, Brown ED, Smith JC Jr. Effects of storage and handling conditions on concentrations of individual carotenoids, retinol, and tocopherol in plasma. *Clin Chem* 1988;34:44-48.
8. Dhariwal KR, Hartzell WO, Levine M. Ascorbic acid and dehydroascorbic acid measurements in human plasma and serum. *Am J Clin Nutr* 1991;54:712-716.
9. Longcope C, Franz C, Morello C, Baker R, Johnston CC. Steroid and gonadotropin levels in women during the perimenopausal years. *Maturitas*; 1986;8:189-196.
10. Mickelson KE, Petra PH. Purification and characterization of the sex steroid-binding protein of rabbit serum. Comparison with the human protein. *J Biol Chem* 1978;253:5293-5298.
11. Longcope C, Hui SL, Johnston, CC. Free estradiol, free testosterone, and sex hormone-binding globulin in perimenopausal women. *J Clin Endocrinol Metab* 1987;64:513-518.