Dietary Effects on Lipoproteins and
Thrombogenic Activity

Manual of Operations
Protocol 1 Version 1.2
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CHAPTER 1

HYPOTHESES AND ENDPOINTS

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1.0 PROTOCOL 1 HYPOTHESES AND ENDPOINTS

1.0.1 Objectives:

The purpose of this study is to determine the effect of reducing total dietary and saturated fat intake on plasma lipids and lipoproteins, and on hemostatic factors. The study will be carried out in healthy male and female, white and black adults. In particular, we are interested in determining if further reductions in total and saturated fat, beyond the levels recommended in the Step 1 diet, are efficacious in these gender and ethnic specific groups.

1.0.2 Specific Aims:

1. To compare the effects of three diets, differing in total and saturated fat content, on plasma lipids and lipoproteins in healthy adults.

2. To compare the effects of these diets on plasma hemostatic factors in healthy adults.

3. To determine if males and females, and individuals with different ethnic origins respond to these diets.

4. To evaluate the role of the menstrual cycle in modulating diet responses in females.

1.0.3 Study Outcomes:

1. The primary lipid and lipoprotein endpoints will be plasma concentrations of total cholesterol and triglycerides, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, apoprotein **B**, apoprotein **A-I**, and lipoprotein (a). In addition, we will determine the apoE genotype of each participant.

2. The primary hemostatic endpoints will be plasma levels of fibrinogen, Factor VII and Plasminogen Activator Inhibitor I (PAI-1).

3. Several ancillary studies will be carried out to study a variety of secondary endpoints.

1.0.4 Rationale for endpoints:

The choice of endpoints derives from the need to minimize the comparisons in order to maintain statistical power, and the feasibility of carrying out numerous measurements in a large population of subjects. The plasma lipid and lipoproteins chosen were deemed to be those most likely to affect risk for cardiovascular disease. In addition, issues related to HDL cholesterol are of particular interest, both from the standpoint of gender-specific responsiveness and the response to increasing reductions in total and saturated fat. The inclusion of lipoprotein (a) is based on its emergence as a significant risk factor and the paucity of information related to its response to dietary perturbations. ApoE genotyping will be performed because of the possibility that it affects diet responsiveness. The choice of hemostatic factors was based on data from epidemiologic studies indicating the significance of fibrinogen, Factor VII and PAI-1 as risk factors for coronary heart disease.

1.1 ORGANIZATION AND ADMINISTRATION

The organizational structure of this study will include the following main components: the Field Centers, the Coordinating Center, the Steering Committee, and an external Protocol Review Committee to provide oversight and advice to the NHLBI at various stages of the study. The structure and function of these study components are described below.

1.1.1 Field Centers

There will be four field centers responsible for the recruitment, feeding and investigation of study participants. Each field center will consist of a team of investigators who will provide the necessary skills and effort to develop and carry out this protocol successfully. The Principal Investigator and designated key co-investigators from each field center will participate in protocol development and in decisions concerning the conduct of the study and the analysis and publication of its results via the Steering Committee and its subcommittees.

1.1.2 Coordinating Center

The Coordinating Center will have primary responsibility for coordinating the efforts of the study investigators and for editing, storing, and analyzing the data generated by the field centers and by central laboratories established for key study measurements. Its' investigators and staff will have a central role in designing the data collection system and in monitoring data quality.

1.1.2.1 Food Analysis Laboratory Control Center (FALCC)

FALCC is the central laboratory for food analysis and associated research; it will receive, composite, assay and archive diet samples from the study.

The FALCC will undertake the following tasks:

1. Develop the necessary protocols for diet sampling, diet composition, and analysis and storage of diet composites.

2. Develop, modify and validate the various analytical methods needed for the assay of the diet composite for the food components of concern.

3. Monitor the diets being fed to verify that they have the nutrient content that is planned for the particular experimental feeding trial, and that the diets being fed at the different centers are virtually identical.

The director will be a member of the Diet Subcommittee and will participate as a non-voting member of the Steering Committee.

1.1.2.2 Nutrient Composition Laboratory (NCL) U. S. Department of Agriculture

The NCL will provide standard reference materials for quality control of the food assays. It will also provide expert consultation on all aspects of the control of diet composition. The Director will be a member of the Steering Committee.

1.1.3 Steering Committee

The Steering Committee is primarily responsible for all decisions pertaining to the design and conduct of the studies undertaken in the DELTA project. It will determine the scientific objectives of the studies, design various studies to attain these objectives -- e.g., this protocol -- and oversee the scrupulous implementation of the study protocols. The voting members of the Steering Committee are the Principal Investigators of each field center and the Coordinating Center and the NHLBI Project Officer. Each has a single vote; a simple majority decides. However, approval of the external Protocol Review Committee will be required for any significant changes in protocol, including the initiation of ancillary studies that are recommended by the Steering Committee during the course of the study. The chairperson of the Steering Committee will be appointed by the Director, Division of Heart and Vascular Diseases, NHLBI.

The Steering Committee will meet at least 6 times during the first year and quarterly in subsequent years. It will designate the following subcommittees of investigators and/or staff as needed to oversee aspects of the study that require more frequent attention and/or special expertise. Each subcommittee will have appropriate representatives from each field center and the Coordinating Center:

1. Protocol Subcommittee: will oversee the development of study protocols for implementing study objectives identified by the Steering Committee. The Protocol Subcommittee will develop study design, sample size calculations, eligibility criteria, data variables and sequencing of measurements. It will review relevant data collections forms, consent forms, and manuals of operation for each study protocol.

2. Diet Subcommittee: will oversee the development and testing of the study diets, identify food composition assays, evaluate nutrient databases, review relevant data collection forms and manuals of operation.

3. Manual(s) of Operation and Forms Subcommittees: will provide editorial assistance and final approval for manual(s) of operation, provide content input for data collection forms, approve design and format of forms, review suggested or required changes to forms and procedures after the start of the study.

4. Laboratory Subcommittee: will advise the Steering Committee on appropriate laboratory measurements to achieve study objectives, and monitor performance of the hemostasis, lipid and lipoprotein laboratories and will oversee training and certification of phlebotomists, and laboratory standardization via reports from the Coordinating Center. This committee will review relevant manual(s) of operation and data forms.

5. Publications Subcommittee: will advise the Steering Committee on publication policy. It will oversee the preparation and review of abstracts and manuscripts emanating from the study to assure proper distribution of analysis topics and authorship credit among study investigators. It will ensure that collaborative manuscripts represent the study accurately.

6. Conflict of Interest Subcommittee: will draft guidelines regarding outside activities of study investigators that represent potential conflicts of interest and collect annual disclosure statements from investigators regarding relevant activities.

   DELTA investigators in cooperation with the NHLBI project office will seek donations of products, equipment and services to support specific activities of the study.

   We will follow the guidelines established by the NHLBI for obtaining third party support.

   A major effort will be made to get food donations from national companies in order to defray the cost of feeding the diets across clinics. Other potential third party support would include cholesterol screening machines and various participant incentives.

7. Ancillary Studies Subcommittee: will review and make recommendations regarding the merit and feasibility of ancillary studies that are proposed by study investigators or other interested parties.

The Steering Committee will retain the prerogative to add, combine, delete, or redefine subcommittees as the study evolves and its needs change.

1.1.4 External Oversight

1. Protocol Review Committee: This committee of at least five experts not otherwise affiliated with the study will be appointed by the Director, NHLBI to review this protocol, to recommend revisions as needed, and to advise the Institute as to its acceptability. This study will not be implemented until the Director, NHLBI, acting with the advice of this committee, approves this protocol.

   This committee will meet prior to initiation of each new study and additionally as necessary to carry out its oversight responsibility.

   The Chairperson of the Steering Committee, Project Officer, and designated NHLBI staff will participate in these meetings in an ex-officio capacity, to facilitate communication between the Protocol Review Committee and Steering Committee.

   The Principal Investigator of the Coordinating Center and designated Coordinating Center staff will attend these meetings (but will not have a vote) and will be responsible for preparing and presenting up-to-date statistical reports on the progress

of the study. These reports will include data on recruitment and randomization, as well as statistical tests and special analyses requested by the committee.

1.1.5 Human subjects protections

A. Informed consent

Informed consent will be obtained from each participant before they are enrolled in the study. A draft Informed Consent form is found in the Appendix to this manual. The consent form will describe the risks and benefits of participating in the study, as well as the responsibilities of the participants and the investigators.

B. Privacy

Privacy in the context of this study includes confidentiality of data and personal information at the field center and in handling and reporting of data by the Coordinating Center (see Chapter 7). It also includes discretion on the part of field center staff and arrangements for physical privacy during interviews and examinations.

C. "Right-to-know"

Information obtained at screening, before enrollment, would be available at that time. Abnormal values found at screening would be reported to the participant and also, upon request, to his or her personal physician. During the course of the study, participants occasionally are curious about their progress and about changes in a variable such as serum cholesterol level. Such data will not be available until the very end of the study, because the protocol calls for batch analysis of all samples collected throughout. Information will be provided at the end of the study to participants who are interested.

D. Safety

The DELTA study will evaluate the effects of various diets on blood lipids, and lipoproteins, clotting parameters and related variables. The diets will have varying composition but will not differ greatly from those consumed by many members of the public. All diets will be nutritionally adequate with regard to calories (they will by designed specifically to prevent weight change). All diets will contain at least 90% of the RDA for women 25-50. Unlike those clinical trials evaluating the efficacy of drugs on disease incidence or symptoms, the experimental treatments for the first DELTA study are not expected to pose any particular risk per se. Individuals for whom the diets are contraindicated because of preexisting conditions (for example, liver disease, kidney disease, clotting disorder) would not be eligible for participation according to defined exclusion criteria.

Food allergies will be inquired about during screening.

The collection of biological specimens and other information carries a small risk. For example, phlebotomy carries a risk of bruising and discomfort. This will be explained to the participant in the consent form.

Food safety is another concern. The DELTA investigators have an obligation to protect participants against illness due to microbiological contamination of food. The DELTA field centers will guard against this through rigorous application of food handling standards appropriate for their particular institution (hospital-based kitchen; other setting). Individuals will be carefully instructed in the handling and storage of any take-home food or meals for and coolers and "blue-ice" will be provided. Time-temperature indicators will be enclosed with instructions not to consume certain foods if the strip indicates that holding temperatures have not been appropriate. The consent form will include an explanation of the participant's obligation to handle take-out food according to instructions they will be given to maintain adequate sanitary conditions.

Safety oversight

Safety concerns will be addressed during protocol review by the NHLBI-appointed committee and by the individual field center IRBs. After enrollment, the Principal Investigators will monitor safety issues continuously and report any problems to the Coordinating Center, which will inform the NHLBI Medical Officer. Each field center will have an assigned Medical Officer to ensure the satisfactory disposition of study-related adverse events (referral to physician, treatment, or decision to drop from study for medical reasons which must be made with P.I.).

MEDICAL OFFICERS:

Columbia:	Dr. Henry Ginsberg Dr. Neil Shachter
Minnesota:	Dr. Aaron Folsom
Penn State:	Dr. W. C. Nicholas
Pennington:	Dr. Donna Ryan

CHAPTER 2

RECRUITMENT

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2.0 RECRUITMENT

Recruitment is a key issue with the DELTA Study. Multicenter recruitment in any clinical trial is difficult; however, because of the nature of this nutrition trial and the expected difficulties, the Coordinating Center would like to share some of the GOLDEN RULES OF RECRUITMENT developed by Dr. Bert Spilker at the University of North Carolina.

2.1 GOLDEN RULES OF PATIENT RECRUITMENT (Spilker, Bert and Cramer, Joyce A., *Patient Recruitment in Clinical Trials.* New York: Raven Press, 1992.)

2.1.1 During the Initiation of a Clinical Trial

1. Obtain adequate personnel for answering telephones, scheduling eligibility visits and conducting each type of screening.

2. Train all personnel appropriately and evaluate their skills. Assure that aggressive approaches to patients are not used.

3. Conduct a cost-benefit analysis of each recruitment method considered and choose the ones that are the most desirable and most comfortable for the staff and institution to implement.

4. Use the telephone screening form and eligibility forms for completing the screening process.

5. Provide feedback to the entire team at your site as recruitment progresses.

6. Discuss your trial with the medical community in the catchment areas before any large media recruitment is initiated. Seek agreement to the recruitment approach considered and also seek cooperation in obtaining referrals.

7. Work problems out of the referral system before it is widely used.

8. Take whatever steps are necessary to implement contingency plans that may have to be used at a later state of the clinical trial (.e.g., visit potential sources of patients and discuss recruitment issues with relevant personnel).

2.1.2 During the Conduct of a Clinical Trial

1. Maintain logs of all patients contacted, screened and enrolled in the trial.

2. Track the patient participation at each site in a multicenter trial on a weekly basis.

3. Monitor the trial's conduct to ensure that potential enrollers are not kept waiting for long periods and are treated with appropriate care and respect.

4. Investigators and their staff must spend sufficient time with all patients and their families to answer all questions and to explain the nature of the trial. This explanation should use whatever educational level the family desires and is able to understand.

5. When the actual rate of patient enrollment is less than the expected rate by a predetermined amount for a predetermined length of time (e.g., below quota), investigate the causes of this issue.

6. If the number of enrollers does not achieve a minimum standard per week or month or other period, then take steps to evaluate the number of patient contacts and screens and also to modify the recruitment strategy for your institution.

7. Investigate each recruitment issue thoroughly to develop a counter plan and to initiate a response.

8. When major problems in recruitment occur, call a meeting of all relevant personnel to address the problem.

9. In modifying the recruitment strategy, alter one method at a time if it is important to know which particular change has an effect on recruitment. Changing two or more methods together will complicate the interpretation of which is responsible for any change observed.

10. Adjust (if necessary) the goals used as targets for patient recruitment.

2.2 RECRUITMENT GOALS

The Dietary Effects on Lipoproteins and Thrombogenic Activity study (DELTA) will recruit healthy men and women between the ages of 22 and 72 years of age. The study population will include 60 percent women to obtain adequate numbers of pre- and post-menopausal participants. Pre- and post-menopausal women will be recruited in approximately equal numbers. Minorities will be recruited by all centers in accordance with their availability to participate. The number of African American study participants expected in the Penn State and University of Minnesota centers is quite small; therefore, the Columbia University center and the Pennington Biomedical Research Center will recruit larger numbers of African American participants to supplement this population group (refer to Table 1).

Table 1 gives an example of possible recruitment outcome. The centers will attempt to recruit roughly equal numbers of pre- and post-menopausal women (f+ and f-, respectively.) In this chapter we will use "Black" to refer to African American participants, and "White" to refer to all other participants in the study. We anticipate that this latter category may include a few Asian and Hispanic participants.

Each DELTA center has experience in recruitment and will approach the recruitment phase using sources, methods and strategies that have been most successful for them in the past. Recruitment will take place over approximately two months time and will include the use of flyers, brochures, advertisements and announcements to attract study participants. In addition, lists prepared from recruitment for previous studies, university employee directories and student and community organizations listings will provide targeted sources for mailing purposes. Recruitment will include a variety of sources to produce a wide range in participant age and cholesterol levels.

Table 1

Assumed Sample Design

Center	Gender	Black	White	Total
Columbia U.	F+	3	4	7
	F-	4	4	8
	M	4	5	9
Pennington	F+	4	4	8
	F-	3	3	6
	M	5	5	10
U. Minn.	F+	1	6	7
	F-	1	7	8
	M	1	8	9
Penn State	F+	1	6	7
	F-	1	6	7
	M	1	9	10
Total	F+	9	20	29
	F-	9	20	29
	M	11	27	38
		29	67	96

M: Males
F+: Premenopausal Females
F-: Postmenopausal Females
Black: African American participants
White: Non - African American participants (mostly Caucasian)

2.3 RECRUITMENT METHODS AND SOURCES BY FIELD CENTER

2.3.1 University of Minnesota DELTA Recruitment

2.3.1.1 Population to be recruited

The University of Minnesota DELTA center will recruit 24 participants who are healthy men and women ranging from 22 to 72 years of age who meet the study eligibility criteria. A special effort will be made to include 3-6 African American participants within the study population of 24 total participants. The recruitment population will include 60 percent women with approximately equal numbers of women being of pre- and post-menopausal status.

2.3.1.2 Sources

Recruitment sources will include lists from previous studies, university employees, age eligible students, and the general population within the surrounding community. Although the minority population in the Twin Cities Area is not large, the University of Minnesota has been successful in recruiting from these groups. Recruitment of African American participants has been enhanced through successful history of recruitment for previous studies and the resulting development of linkages within church and community groups. Care will be taken to target recruitment toward individuals to whom such a study is feasible in an effort to reduce the overall recruitment cost and produce a yield of participants who are likely to be retained throughout the diet periods.

2.3.1.3 Methods and Strategies

The University of Minnesota has successfully recruited participants for feeding studies through informational advertising on the campus. Flyers, posters, employee and student letters and advertisements in the campus newspapers and newsletters will be the methods utilized to attract interest in this feeding trial among the campus population. Recruitment of the surrounding community will involve mailings to specific zip code areas, posters in community locations and advertisements and feature articles in targeted community newspapers.

Strategies will be used to promote early identification of those individuals who are most likely to be eligible. These strategies are designed to reduce staff time and laboratory costs during the recruitment phase and to minimize study drop out rates. Individuals responding to recruitment efforts will be scheduled for a telephone screening interview which will 1) identify the recruitment source, 2) provide a brief overview of the basic feeding study expectations and commitment and 3) screen participants who have disease conditions and/or use medications or special diets that exclude their participation.

2.3.2 Columbia University DELTA Recruitment

2.3.2.1 Population and Sources

First year recruitment will be from two pools - healthy male and female students, and healthy male and female employees. The students are aged 20-30, numbering

about 200 in the first two years at the medical school, about 100 in the first two years at the dental school and about 40 nursing students. There are equal numbers of men and women in all groups except nursing students, who are 3:1 women to men. The racial makeup is 70 percent Caucasian, 10 percent African American, 10 percent Asian and 10 percent other. Male employees are approximately 500 in number in the security and engineering divisions. Almost all are African American/Hispanic and 30-50 years of age. Female employees are from any department at the medical center and number over 500 between the ages of 30-65.

2.3.2.2 Methods and Strategies

We will recruit students, when they return to school in August, through direct mailings (we can add a notice to the information packet to new students from the Dean's office) and posters. During the two weeks before school, when all students are moving in and being oriented, non-fasting, fingerstick cholesterol screening will be offered. This approach will provide an initial pool of about 300 students, half female. As for the employees, the directors of security as well as the director of engineering have agreed to help in recruitment. The men and women work full time at the campus and can eat one meal during work and one meal either before or after work. Many of them live near the campus. The benefits available to the participants, including free physicals and blood analyses, and the provision of free food for several months, will aid in recruitment.

2.3.3 Pennington Biomedical Research Center DELTA Recruitment

2.3.3.1 Population to be recruited

It is the intention of the PBRC Field Center to recruit a minimum of 24 participants between the ages of 22 and 72 who will meet the eligibility requirements. The PBRC Field Center will set as recruitment targets a participant population consisting of 50% Caucasian and 50% African American men and women. Recruitment targets by gender will be 40% men and 60% women. Pre- and post-menopausal women will be recruited in approximately equal numbers.

2.3.3.2 Sources

The PBRC Field Center will recruit participants from the following sources:
PBRC Volunteer Data Base. Over the course of the last 18 months, the PBRC clinic has received over 2,000 responses from individuals wishing to participate in ongoing clinical studies. Approximately 750 individuals participated in on-site screenings, of which 360 were enrolled in one of 9 different clinical studies. In excess of 150 individuals have expressed a specific interest in participating in dietary studies.

Louisiana State University. The LSU campus in Baton Rouge is located within 4 miles (15 minute drive) of PBRC. The campus population is comprised of 22,000 undergraduate students, 5,000 graduate students and 5,000 employees. Many of the students and employees of LSU live in the community immediately surrounding the PBRC Field Center.

Southern University. The Southern University Campus is located approximately 20 minutes away from the PBRC Field Center. Southern University is an historically African American university and currently has an enrollment of over 9,000 students. The PBRC has an established record of interaction with Southern University.

Surrounding Baton Rouge Community. To assure adequate participant volunteers, the PBRC Field Center has cultivated a constituency among the Baton Rouge community including ties to its civic, social and religious organizations.

2.3.3.3 Methods and Strategies

The following methods and strategies will be employed to recruit participants: All recruiting will be coordinated through a full-time Clinical Subject Recruiter. A Minorities Subject Recruiter will join the clinic staff this summer to facilitate recruitment in the African American Community. Advertisements describing the study will be placed in the university and community newspapers.

Publications Director, Ben Phillips, a veteran journalist, will contact local newspapers and the radio and television stations to publicize the study and volunteer recruitment. Public Education Director, Ruth Patrick, Ph.D., performs a weekly five minute spot on a local television station on nutrition-related issues. Her presentation of research activities at the PBRC will be used to aid in participant recruitment.

2.3.4 Penn State University DELTA Recruitment

2.3.4.1 Population to be recruited

The Pennsylvania State University DELTA center will recruit 24 healthy men and women, aged 22-72 who meet the study eligibility criteria. Three black participants will be recruited (see Table 1). The study population will include 60 percent women of which an equal number of pre- and post-menopausal women will be recruited.

2.3.4.2 Sources

Recruitment sources will include students (there are over 4,000 age-eligible students on the University Park campus - mostly graduate students) and the general population within the surrounding community. To target postmenopausal women, we will work with the University's Office of Health Promotions (OHP). Over 1,000 women (mostly clerical staff) over the age of 50 have participated in recent health programs and have expressed interest in continued participation. We will work with the OHP's director, who indicates that this is a health-conscious and motivated group. Thus, we will recruit specifically from this pool.

2.3.4.3 Methods and Strategies

The Penn State DELTA center has developed effective recruitment strategies for use in previous studies; these include word-of-mouth and announcements to graduate student organizations, newspaper advertisements (both campus and local papers), newsletter articles,

radio announcements and personal letters. Lists and mailing addresses of graduate students will be generated from the PSU registrar. In addition, letters will be mailed to university employees who have participated in past programs held through the OHP.

Respondents will be screened initially by telephone and through recruitment meetings. Postmenopausal women will be scheduled for a telephone screening interview. Recruitment meetings, which are held at the DELTA study facilities, have been used by us in the past and are very efficient for mass-screenings of college students. Interested individuals are directed to an information meeting, held on several different dates and times, to learn more about the study. We find that very interested and highly motivated individuals attend the meetings, and that holding the meetings in the facility allows potential participants to visualize their involvement in the study. We also find that this is another indirect method of recruitment, since people often come to these meetings in pairs or groups (a buddy system). Following the meeting, interested individuals will be scheduled for a formal screening visit.

2.4 RECRUITMENT FORMS

A recruitment source log is used by the DELTA centers to record all methods utilized to recruit participants. Each method used receives a code. Prospective participants will be asked to indicate where their knowledge of the study originated, and the appropriate code will be included on their eligibility forms.

2.4.1 Recruitment Source Log

The recruitment source log would be used to record any and all methods employed to recruit participants. Each method used would receive a code and additional information would be recorded (including where a poster, flyer, article or advertisement was placed, the date, and the cost).

The code is derived using a three digit system where the first digit would indicate the major method category (i.e., written materials, network publicity, mailings or word of mouth), the second digit could indicate the method within a category (i.e., flyer, poster, newspaper advertisement, etc.) and the third digit could be center-specific for tracking of the response rate on a particular poster, advertisement, etc.

The shaded areas to the right of the form allow information regarding number of responses generated and actual number of scheduled visits resulting from a particular method to be recorded.

The log, itself, would not require data entry. It would serve as a record of recruitment methods and sources used. The assigned code would be major importance as a descriptor of the recruitment method and source utilized at the field center. Each center will be required to report recruitment methods and sources utilized at their center. Also, copies of each recruitment material will be sent to the Coordinating Center for archival purposes. This information will not be entered into the Data Management System (DMS); however, the Coordinating Center will take the raw data sent to the Coordinating Center and report results.

Prospective participants would be asked to indicate where their knowledge of the study originated on the Telephone Screening Form.

Because of the different recruitment methods and different populations at each site, it is felt that a multicenter approach cannot be taken with this. Each site will be able to generate cost comparisons on method and yield for each center-specific site. Information gained would be extremely valuable in the ongoing recruitment process for Protocol 1 and will be used to target recruitment efforts to improve yield and cost effectiveness.

Written Materials Method	Code	Date	Additional Information	Cost	#Resp	#Sched
Flyers						
1.						
2.						
3.						
4.						
5.						
Posters						
1.						
2.						
3.						
4.						
5.						
Newspaper Advertisements						
1.						
2.						
3.						
4.						
5.						
Newsletter Articles						
1.						
2.						
3.						
4.						
5.						
Other						
1.						
2.						
3.						
4.						
5.						

Network Publicity Method	Code	Date	Additional Information	Cost	#Resp	#Sched
Radio PSA						
1.						
2.						
3.						
4.						
5.						
Radio Talk Show						
1.						
2.						
3.						
4.						
5.						
Television PSA						
1.						
2.						
3.						
4.						
5.						
Television Talk Show						
1.						
2.						
3.						
4.						
5.						
Other						
1.						
2.						
3.						
4.						
5.						

Mailings Method	Code	Date	Additional Information	Cost	#Resp	#Sched
Existing Lists						
1.						
2.						
3.						
4.						
5.						
Check Stuffers						
1.						
2.						
3.						
4.						
5.						
Department Mailboxes						
1.						
2.						
3.						
4.						
5.						
Other						
1.						
2.						
3.						
4.						
5.						
Word of Mouth						

CHAPTER 3

VISIT SCHEDULE AND DESCRIPTION OF ELIGIBILITY VISITS

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3.0 VISIT SCHEDULE AND DESCRIPTION OF ELIGIBILITY VISITS

There are three encounters with the applicant (Telephone Screen, Eligibility Visit 1 and Eligibility Visit 2) where eligibility is determined. The schedule of these visits and an outline of important features and requirements of these visits are in this chapter. Instructions for completing all data collection forms are found in Chapter 7.

All items on the study forms which require medical review must be reviewed by medical personnel before proceeding. Forms should NOT be completed retrospectively from clinic notes or memory. If corrections are made to the form after the data are eventually keyed into the Data Management System (DMS), give the form to the person who is responsible for keying data at your site to make the necessary corrections.

SCHEDULE

Screening Episode	Timeframe
Telephone Screening Form	Anytime
Eligibility Visit 1 (EV1)	At least one week after initial contact from potential participant
Eligibility Visit 2 (EV2)	One to four weeks after EV1

3.1 TELEPHONE SCREENING

3.1.1 Purpose

1. To introduce the study and answer questions the applicant may have.
2. To have the interviewer complete the Telephone Screening Form.
3. To determine initial eligibility and complete general medical condition questionnaire.
4. To determine alcohol consumption.
5. To obtain a list of current medications.
6. To obtain weight and height and compare against upper weight limit table.
7. To determine if women in child-bearing age are pregnant, planning to become pregnant, breastfeeding or have borne a child within the last 6 months.
8. To give a general description of the study and determine further interest.
9. To schedule EV1 if applicant remains eligible.

3.1.2 Items Needed for Telephone Screen

1. TSF questionnaire to be completed by the interviewer.

3.2 GENERAL INSTRUCTIONS

Please use the following guidelines when completing any of the data collection forms:

1. Use a black ball-point pen and **print** neatly.

2. List all dates numerically as month/day/year, e.g. March 21, 1961 is 03/21/61.

3. Data collected on April 3, 1993 would be entered as Today's Date: 04/03/93

   NOTE: DELTA usually records time using a 12-hour clock, with AM or PM indicated separately. Colons (":") are used as the separator character for hours and minutes. The format to be used is indicated under the space. Use leading zeros within each time unit (hour or minute) so that each space is filled. Note that midnight is recorded as 12:00 AM, and noon is recorded as 12:00 PM.

   Example: A time of fasting determination of 8:05 in the morning is entered as:

   a. Time: 08:05 b. AM (A) or PM (P): A

4. Make corrections by drawing a single line through the incorrect entry and then indicate the correct entry as near to the incorrect entry as possible. Initial and date the correction. **DO NOT USE CORRECTION TAPE OR FLUID.**

5. If data requested are not applicable to the applicant, please indicate by NA (Not Applicable). If data requested are not known or if particular procedure was not performed, please enter ND (No Data).

   The following forms should be completed at the field centers at the time indicated:

Form	Time for completion
Telephone Screening Form (TSF)	Phone interview
Eligibility Visit 1 Form (EV1)	Visit 1
Eligibility Visit 2 Form (EV2)	Visit 2
Baseline Lipid Profile Form (LIP)	Visit 2
Additional forms to be completed include:	
Participant Agreement Form	
Consent Form	

6. Read all forms instructions and the actual forms prior to beginning an interview. This will greatly enhance flow of the interview and the accuracy of the information collected.

7. **Always** complete the applicant ID number, your code number, and the date the form is being collected first. This information is requested at the beginning of each form.

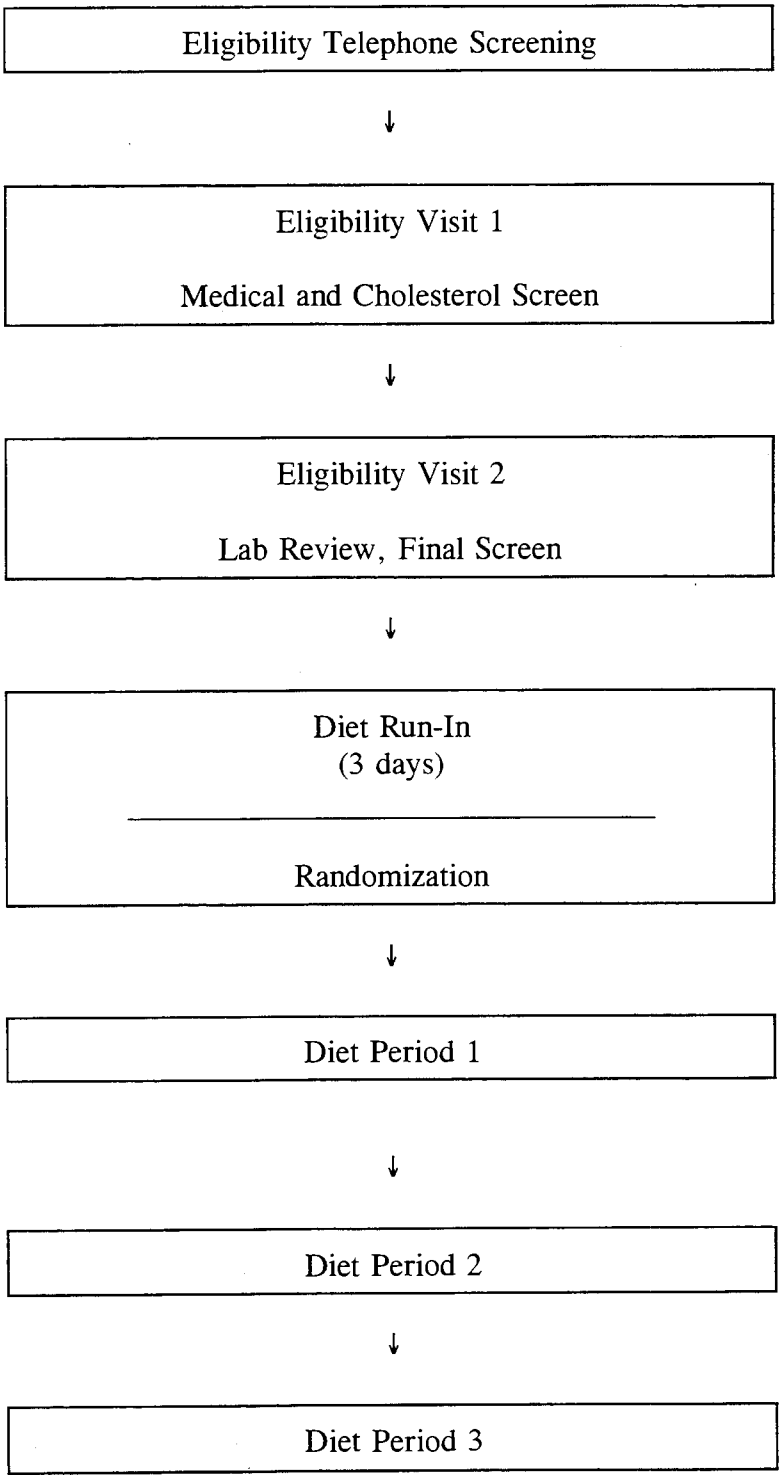
8. Ask the questions exactly as they are written on the forms.

9. Please retain all the participant data for at least 5 years following the completion of the trial. Before destroying any records, please contact the DELTA Coordinating Center.

DO NOT HESITATE TO CALL, (919)-962-3092 IF YOU HAVE ANY QUESTIONS REGARDING THESE FORMS!

3.3 VISIT FLOW CHART

DELTA Protocol 1 Design Overview - Visit Sequence



3.4 TELEPHONE SCREENING FORM (TSV)

GENERAL REMINDERS: Please use black ball-point pen. Print all responses legibly. Initial and date all corrections. Enter data in ALL spaces provided. Enter NA or ND where applicable (see GENERAL INSTRUCTIONS SECTION for complete instructions).

Attach the applicant's ID label to the space provided.

Questions 1-2: Date of data collection and code number of person completing the form

1-2. Enter today's date at the top of the page and your code number.

Questions 3-6: Applicant Name, address, home telephone number and work telephone number

3-6. Enter the applicant's complete name, address, home telephone number and work telephone number. Please ask for correct spelling if necessary. Read this information back to the applicant to ensure accuracy.

Questions 7-10: Demographics

7. Enter birth date numerically as month, day and year. For example, March 14, 1965 should be entered as 03/14/65.

8. Enter the applicant's age in years in the space provided.

9. If uncertain, ask applicant what is their gender and indicate as M (male) or F (female) in the space provided.

10. It is possible that some applicants may be offended by the next question, "What do you consider your race to be?" Indicate letter representing response in the space provided. For example, for Caucasian indicate **(A)**. **Please note** "Did not respond" (M) is an option for this answer.

Question 11: Advertisement of Study

a. Indicate letter representing response in space provided. For example, for radio PSA (Public Service Announcement), indicate E in the space provided.

b. Responses not included here can be listed under "other" (N). Please indicate briefly the applicant's response in the space provided.

Questions 12-13: Administrative Information

This section is to be completed at the end of the telephone interview. At this point in the interview, proceed to question 14 under, Medical Conditions. Return to this section at the end of the interview and follow the instructions below.

12a. Indicate for question 12a whether applicant is eligible for the study.

If any medications are listed under items 21-26, they must be reviewed by medical personnel before a decision can be made about the applicant's participation. Please check the accuracy of the applicant's telephone number and request a convenient time when they can be contacted regarding eligibility for the study and possible scheduling for their first eligibility visit. Record this information under question 5.

b, c, d. If applicant is eligible for the study at this time, schedule a date and time for screening visit 1. Please remind applicant to bring all prescription and non-prescription medications, including oral contraceptives, to the first visit.

There may be other reasons for exclusion from the study than those indicated by particular question numbers on the Telephone Screening Form, Eligibility Visit 1 Form, or Eligibility Visit 2 Form. The following 3-character codes can be entered in these fields for the reasons stated:

Code Reason for Exclusion

X11 Applicant was eligible but did not come to next visit.

Action for Question 13a:

Circle A for Telephone Screening if person was eligible following the telephone screening visit but did not come to eligibility visit 1.

Circle B for Eligibility Visit 1 if person was eligible following eligibility visit 1 but did not come to eligibility visit 2.

Circle C for Eligibility Visit 2 if person was eligible following eligibility visit 2 but did not come to run-in.

X12 No interest in this person for this protocol.

X13 Value(s) from applicant's baseline blood sample at eligibility visit 2 is outside eligible range for applicant's gender, race, and age.

Question 14: Medical Exclusions

1. For question 14, read the entire list of medical exclusions to the applicant and circle Y for yes, N for no **or** never tested, and U for unsure.

2. Listed below are a number of medical terms associated with certain diseases which the applicant may note when answering this question. This list is meant to help you complete this form accurately. It is **not** a comprehensive list.

Heart disease: myocardial infarction, coronary occlusion, coronary thrombosis, or congestive heart failure.

Diabetes: sugar in the blood or urine

High Blood Pressure: hypertension, high blood

Renal Disease or Kidney disease: nephritis, pyelonephritis, glomerulonephritis,chronic kidney infections

Gastrointestinal Condition: Crohn's disease, irritable bowel syndrome, ulcer problems, ulcerative colitis, acute ulcer, gastric resection, bowel surgery

Cancer

Blood Clotting Disorders

Liver Disease: cirrhosis, alcoholic liver disease, chronic hepatitis

Conditions that require steroid medication: asthma, allergies

Gout requiring treatment: if applicant has been told in the past that they have gout but they are not taking any medication for this problem, they are still eligible for the study.

Sickle Cell Anemia: this does **not** include sickle cell trait

If any of the items in question 14 are answered yes, the applicant has become ineligible for the study. Please thank the applicant for his/her interest in the study and end the interview.

Question 15: Other Medical Conditions

Enter Y for yes or N for no. If yes, list the medical condition noted. Please attempt to obtain the technical term for the medical condition rather than a common term, e.g. hypertension or high blood pressure instead of "high blood". This section should be reviewed by medical personnel if Y is entered.

Question 16: Food Allergies

Enter Y for yes and N for no. If yes, please attempt to obtain as complete information as possible, e.g. if milk is noted, please ask if allergic to all dairy products; if nuts are noted, please ask if allergic to all nuts or just certain types.

Question 17: Special Diets

Enter Y for yes and N for no. If yes, ask the applicant if it is for diabetes, heart disease, hypertension/high blood pressure, or renal/kidney disease and circle Y for yes, N for no, or U for unsure. If the applicant is on a special diet other than these diets, simply enter N for all responses and proceed to question 18.

Question 18: Other Special Diets

Ask the applicant if they are on any other diet aside from or in addition to the diets listed above. Enter Y for yes and N for no. If yes, list the name of the diet or diets in the space provided.

Question 19: Alcohol Consumption

1. Enter Y for yes and N for no.

2. If Y, enter the number of drinks the applicant consumes in a seven day week weekdays and weekends together). Note: 1 drink= 1, 5 oz. glass of wine; 1, 12 oz. serving of beer; or a 1 oz. shot of liquor.

Questions 20-26: Medications

1. Enter Y for yes and N for no.

2. If Y, under items 21-26 enter all medications taken, either prescribed by a doctor or self-prescribed. Ask applicant for correct spelling. If possible, have applicant read spelling directly off of the product label. For each entry, indicate whether the medication is prescribed by a doctor by circling Y or N. Be sure to list the reason for taking the medication.

Reminder: If any entries are made to this section, it must be reviewed by medical personnel before you can determine if the applicant is eligible to participate in the study.

Questions 27-29: Body Mass Index (BMI)

1. Enter the applicant's height in feet and inched or meters. Height should be without shoes rounded down to the nearest inch or centimeter.

2. Enter applicant's weight in pounds or kilograms.

3. For question 29, enter Y for yes and N for no. If the applicant indicates no, let them know that this study requires participants **not** to lose or gain weight.

4. Find the applicant's height in the BMI table provided. If their weight exceeds the weight listed below their height by at least 5 pounds (or 11 kg), they are ineligible for the study. Thank him/her for their time and interest and end the interview.

Questions 28-29: Women born after 1942 only

Enter Y for yes and N for no.

At this point in the interview, if the applicant remains eligible for the study, read them the general description of the DELTA study.

Question 30: Frequency of Visits

Enter Y for yes and N for no. This is an important question. The applicant must be able to come to the center to eat two meals, 5 days a week.

Question 31: Further Interest

1. Enter Y for yes and N for no.

If Y and the applicant is eligible and will to come to the center to learn more about the study, have a cholesterol and blood pressure screen, schedule them for eligibility visit 1. **Enter this information under question 12 of this form.**

2. If N, circle one reason why the applicant can not participate in the study and **complete question 12 of this form.**

DELTA ID: _____ 1. Today's Date: _____ (mm/dd/yy) 2. Personnel Code Number: ____

3. _____
   a. First Name b. MI c. Last Name

4. _____
   a. Street

   _____
   b. City c. State d. Zip Code

5. Home Telephone: _____ (area-###-####) 6. Work Telephone: _____ (area-###-####)
   When is the best time to call? _____

Can you receive a call at work? YES NO

7. Date of Birth: _____ 8. Age: _____ 9. Gender: ___ (M/F)

10. a. What do you consider your race to be? [Circle letter preceding your selection]
    - A Caucasian (white)
    - B African American (black)
    - C Hispanic
    - D Mixed Race
    - E Chinese
    - F American Indian
    - G Asian Indian
    - H Korean
    - I Vietnamese
    - J Japanese
    - K Pacific Islander
    - L Other
    - M Did not respond

    (b. describe: _____)

11. a. How did you hear about this study? [Circle letter preceding your selection]
    - A Flyer
    - B Poster
    - C Newspaper Ad
    - D Newsletter Clip
    - E Radio PSA
    - F Radio Talk Show
    - G TV PSA
    - H TV Talk Show
    - I Letter by Mail
    - J Flyer in Paycheck
    - K Letter in Dept. Mailbox
    - L Physician or Nurse
    - M Word of Mouth
    - N Other

    (b. describe: _____)

[Complete this section after telephone screening interview.]

12. a. Is applicant eligible following telephone screening? YES NO NEEDS MEDICAL REVIEW (R)

    b. If YES to 12a, date of Eligibility Visit 1: _____ (mm/dd/yy) c. Time: _____ (hh:mm) d. AM PM

    e. If NEEDS MEDICAL REVIEW to 12a, does the applicant remain eligible? YES NO

13. a. If this applicant has been excluded from the study, at which time point was the applicant excluded: [Circle letter preceding selection]
    - A Telephone Screening
    - B Eligibility Visit 1
    - C Eligibility Visit 2

    Indicate reasons for exclusion: [Enter question number of exclusion, e.g. 14E or 32, left-justified] b. _____ c. _____ d. _____

MEDICAL CONDITIONS

14. Because certain medical conditions will interfere with our study, we need to ask the following questions. Do you have any of the following medical conditions? [Read list of medical conditions and circle response YES (Y), NO (N) if NO or NEVER TESTED, or UNSURE (U)]

a. heart disease	Y	N	U
b. diabetes	Y	N	U
c. high blood pressure or hypertension	Y	N	U
d. renal or kidney disease	Y	N	U
e. gastrointestinal condition (Crohn's disease, irritable bowel syndrome, ulcer problems, bowel surgery)	Y	N	U
f. history of blood clotting disorders	Y	N	U
g. liver disease (cirrhosis)	Y	N	U
h. condition that requires steroid medication	Y	N	U
i. gout requiring treatment	Y	N	U
j. recent history of depression or mental illness requiring medication within last 6 months	Y	N	U
k. anemia	Y	N	U
l. sickle cell anemia	Y	N	U
m. lung disease, chronic bronchitis, emphysema	Y	N	U
n. acquired immune deficiency syndrome (AIDS) or positive HIV test	Y	N	U
o. cancer (active within 5 years)	Y	N	U

[If any medical condition was circled UNSURE, or item J was circled YES, then review by medical personnel is required to exclude the applicant from participation. If any item, other than J, was circled YES, then the applicant has become ineligible. If so, terminate the interview and complete questions 12-13.]

OTHER MEDICAL CONDITIONS

15. a. Do you have any other medical conditions not listed above? YES NO

If YES, list other medical conditions, one per line:

b. _____

c. _____

d. _____

[If any medical condition is listed, review by medical personnel is required.]

FOOD ALLERGIES

16. a. Do you have any food allergies? YES NO

If YES, list allergies, one per line:

b. _____

c. _____

d. _____

[If any food allergy is listed, review by medical personnel is required.]

SPECIAL DIETS

17. Are you on a special diet prescribed by a doctor for a medical condition? YES NO

If YES, is it: [Read list of special diets and circle response YES, NO, or UNSURE]

a. diabetes	YES	NO	UNSURE
b. heart disease	YES	NO	UNSURE
c. hypertension or high blood pressure	YES	NO	UNSURE
d. renal or kidney disease	YES	NO	UNSURE
e. any other disease	YES	NO	UNSURE

[If any special diet is circled YES or UNSURE, review by medical personnel is required.]

ALCOHOL CONSUMPTION

DEFINITION: 1 drink = a 5 oz. glass of wine, a 12 oz. can of beer, or a shot glass of liquor

18. a. Do you drink alcoholic beverages? YES NO

b. If YES, how many drinks do you usually have in a 7-day week? _____

[If the applicant usually drinks over 12 drinks in a 7-day week, then the applicant has become ineligible. If so, terminate the interview and complete questions 12-13.]

MEDICATIONS

19. Do you take any type of medication? YES NO

If YES, what is the name of the medication that you take? [Record both doctor prescribed and self-prescribed medications. Ask for spelling of medication if necessary.]

a. Medication	b. Prescribed by a Doctor YES(Y) or NO (N)	c. Reason for Taking Medication
20.	Y N	
21.	Y N	
22.	Y N	
23.	Y N	
24.	Y N	
25.	Y N	

[If YES was circled for any medication prescribed by a doctor, review by medical personnel is required.]

HEIGHT AND WEIGHT

[Choose whether you will enter height/weight in customary units (ft-in/lb) or metric units (cm/kg) and proceed to the questions following the appropriate Upper Weight Limit Table. Only enter responses for either questions 26-28 or 29-31.]

Upper Weight Limit Table in Customary Units (ft-in/lb)

Ht.	4'10"	4'11"	5'0"	5'1"	5'2"	5'3"	5'4"	5'5"	5'6"	5'7"
Wt.	153	158	164	169	175	181	186	192	198	204

Ht.	5'8"	5'9"	5'10"	5'11"	6'0"	6'1"	6'2"	6'3"	6'4"
Wt.	210	217	223	229	236	243	249	256	263

26. What is your height without shoes? a. ft: _____ b. in: _____

27. a. What is your weight without shoes? lbs: _____
b. Is the applicant's weight recorded in question #27a greater than the upper weight limit for the applicant's height in the table above? YES NO

28. It is important that our participants not lose or gain weight in this study. Are you willing to participate in a study where your weight is maintained at the same level it is now? YES NO

[Proceed with instructions at the bottom of the page.]

Upper Weight Limit Table in Metric Units (cm/kg)

Ht.	148	15	152	15	156	15	16	162	16	166	168	170
Wt.	70	72	74	76	78	80	82	84	86	88	90	92

Ht.	17	17	17	178	180	182	184	186	188	190	19	194
Wt.	95	97	99	101	104	106	108	111	113	116	11	120

29. What is your height without shoes? cm: _____

30. a. What is your weight without shoes? kg: _____
b. Is the applicant's weight recorded in question #30a greater than the upper weight limit for the applicant's height in the table above? YES NO

31. It is important that our participants not lose or gain weight in this study. Are you willing to participate in a study where your weight is maintained at the same level it is now? YES NO

[Proceed with instructions at the bottom of the page.]

[If the applicant's weight is greater than the upper weight limit, or the applicant is not willing to maintain the same weight during the study, then the applicant has become ineligible. If so, terminate the interview and complete questions 12-13.]

WOMEN BORN AFTER 1942 ONLY

32.	Are you pregnant or planning to become pregnant within the next year?	YES	NO
33.	Are you breastfeeding?	YES	NO
34.	Have you had a baby within the last 6 months?	YES	NO

[If applicant remains eligible, continue with general description of DELTA Study.]

Description of DELTA Study

The purpose of the DELTA Study is to compare the effects of 3 diets with different amounts of fat on the level of cholesterol and clotting factors in the blood. The length of the study is 8½ months. Participants will eat each diet for 8 weeks. There are breaks between the diet periods, including time off for Thanksgiving, Christmas, New Year's, Passover, and Easter holidays.

During the diet study, you will be required to eat only foods provided by the study staff. You will not be allowed to eat any other food. You will be required to eat two meals at the center each day, Monday through Friday. The other meal will be packaged at the center and taken out. Weekend meals will be packaged also. Extra foods for snacks will also be provided. You will be allowed a meal of your own choice once a week, which will be Saturday dinner.

The diets will be planned so that you do not gain or lose weight during the study.

During weeks 5, 6, 7, and 8 of each diet period, blood samples will be taken to provide research information on cholesterol, other blood fats, and blood clotting factors.

FURTHER INTEREST

35. Based on the description of the study I just read to you, would you be interested in coming to the center to learn more about this study and get your cholesterol and blood pressure checked? YES NO

If NO, what is the reason? [Circle response to reasons for not scheduling Eligibility Visit 1]:

a. Uninterested in general study protocol YES NO

b. Unwilling to commit due to length of study YES NO

c. Unwilling to come to feeding center for 2 meals each day for 5 days each week YES NO

d. Unwilling to eat study food YES NO

e. Unwilling to limit intake to study foods only YES NO

f. Unwilling to allow maintenance of current body weight YES NO

g. Lives too far from feeding center YES NO

h. Travels out of town as part of job position YES NO

i. Other (j. specify: _____) YES NO

[If applicant is not interested, terminate the interview and complete questions 12-13.

If applicant is interested and remains eligible to this point, then go to question 36 and schedule Eligibility Visit 1. Ask applicant to bring all medications, including diet supplements and any contraceptives, to Eligibility Visit 1.

If applicant is interested, but responses need medical review, then tell applicant that he/she will be called back. Go to question 37 and schedule time for callback.]

36. If applicant is eligible at this point, schedule Eligibility Visit 1: a. date: _____ (mm/dd/yy) b. time: _____ (hh:mm AM/PM)

37. Does applicant's responses need medical review? YES NO

If YES, when is the best time to call? _____

[Return to page 1 and complete questions 12 and 13.]

3.5 ELIGIBILITY VISIT DESCRIPTION AND DATA FLOW

1. Prepare the EV1 and EV2 items before the applicant arrives. Place labels on all the forms.

2. Greet the applicant and give a brief description of what will be done at this visit. Answer any questions he/she may have. Collect all the medications brought in at EV1 and ask the participant if he/she has left any medications at home.

3. Give the applicant the consent form before he/she begins Part 1 of EV1. Have him/her read it and sign on the bottom. The signing must be witnessed by one other person. Have the witness sign the consent form after the applicant has signed.

4. All forms should be reviewed for completeness by the study coordinator and given to the data entry person for further editing when the DMS is made available.

5. Page 1 of the Telephone Screening Form will only be entered for those applicants who are randomized into the study.

6. All pages of EV1 and EV2 forms will be entered for all applicants who are randomized into the study.

3.5.1 Eligibility Visit 1 (EV1)

3.5.1.1 Purpose

1. To answer additional questions the applicant may have about the study.

2. To have the applicant complete Part I (Questions #2-23) of the EVI Form.

3. To have the interviewer complete Part II (Questions #24-60).

4. To determine continued eligibility and complete general medical condition questionnaire.

5. To determine alcohol consumption and rule out people who drink more than 12 drinks in a week.

6. To obtain a list of current physician-prescribed and self-prescribed medications.

7. To measure weight and height and compare against upper weight limit table.

8. To determine if there are any medical, personal, or professional reasons that would keep an applicant from participating in DELTA.

9. To determine women's pre-menopausal and post-menopausal status.

10. To measure blood pressure.

11. To measure cholesterol by fingerstick method (using Cholestech machine).

12. To schedule EV2 if applicant remains eligible.

3.5.1.2 Items Needed for EV1

1. EV1 questionnaire to be completed by the interviewer.

2. Blood pressure cuff.

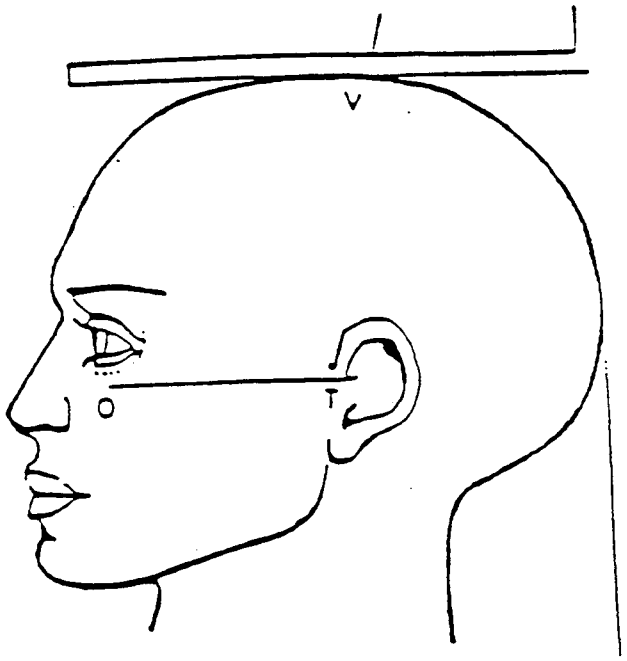
3. Cholestech Machine and cassettes.

4. Collect medications from applicant to verify medicines.

3.6 PHYSICAL MEASUREMENTS

3.6.1 Standing Body Height

The applicant stands erect on the floor or the horizontal platform with his/her back against the vertical mounted metal centimeter ruler, heels together and against the vertical ruler, looking straight ahead with his/her head in the Frankfort horizontal plane (the horizontal plane which includes the lower margin of the bony orbit -- the bony socket containing the eye -- the most forward point in the supratragal notch -- the notch just above the anterior cartilaginous projections of the external ear) (**Figure 1**). The right angle is brought down snugly but not tightly on the top of the head. A foot stool is used if the examiner is shorter than the applicant such that the examiner's view is level with the point of measurement on the head of the applicant. The applicant's height is recorded to the centimeter or inch, rounding down.



ORBITALE:	Lower margin of eye socket
TRAGION:	Notch above tragus of ear or at upper margin of zygomatic bone at that point
FRANKFORT PLANE:	Orbital-tragion line horizontal

Figure 1. Frankfort Plan or Measuring Body Height

BODY WEIGHT SCALES CALIBRATION FORM

A) **DAILY:** At the beginning of each day, scales should be checked to see that they read zero when there is no weight on them.

B) **WEEKLY:** Calibrate the scales using the 50 pound known weight. This calibration is performed again whenever the scales are moved. If the scales are outside the 49.5 to 50.5 range an independent service technician is called in to recalibrate the scales. Calibration with the 50 pound weight is performed for both balance arms (light and heavy) on the scale.

C) **ANNUALLY:** The scales are certified annually by an independent scale technician.

The Anthropometry Equipment Calibration Form should be filled out and sent to the Coordinating Center bi-weekly.

1.	Calibration Check of Scales with 50 lb weight	Initials: _____
	Reading of scales with 50 lb weight	_____
	If reading is outside of 49.5 to 50.5 range, scale should be serviced.	
	If service is requested, give	Date ___-___-___
	Recalibration by independent service technician	Technician: _____
2.	Repeat Calibration because of moving of scales	
	Scales moved:	1. Date ___-___-___ Initials _____ 2. Date ___-___-___ Initials _____
	Calibration:	1. Date ___-___-___ Initials _____ 2. Date ___-___-___ Initials _____

Today's Date ___-___-___

Completed by _____

Center _____

3.6.2 Body Weight

Before an applicant is weighed, the scale is balanced so that the indicator is at zero when no weight is on the scale. The scale must be level and on a firm surface (not a carpet). The applicant is instructed to stand in the middle of the platform of the balance scale with head erect and eyes looking straight ahead. Adjust the weight on the indicator until it is balanced. Record the results down to the pound or kg, rounding down. To maintain accuracy, the scale is zeroed daily and must be calibrated with a known weight (50 lbs.) every week or whenever the scale is moved.

3.6.3 Blood Pressure

3.6.3.1 Cuffs and Bulbs

Proper size of the cuff is essential for accurate blood pressure measurement. Field Centers have four standardized cuffs available - small adult, regular adult, large adult, and thigh cuff.

The range markings on commercial cuffs overlap from size to size and do not offer a precise guideline. In the DELTA study arm size is measured, and the cuff size is selected as follows:

Table 1. Determination of cuff size based on arm circumference

Cuff	Size	Arm Circumference
Small	Adult/Child	<24.5 cm
Regular	Adult	24.5-33.0 cm
Large	Adult	33-40 cm
Thigh		>40 cm

3.6.3.2 Blood Pressure Measurement Instructions

The Coordinating Center is presenting the ARIC procedure at this time in lieu of any final decisions on blood pressure measurements as endpoint determinations (pending Steering Committee decision). Some of the many extraneous factors influencing blood pressure are controlled by standardizing the measurement technique and the environment in which the measurement is made. Blood pressures should be taken before any attempts to draw blood.

3.6.3.3 Staff Preparation for Participant Visit

In relating to the DELTA applicants, remember that participation in the study is voluntary. Applicants are given full explanation and instructions about the preparation for the blood pressure examination and an opportunity for questions. The setting in which blood pressure measurements are made is standardized and takes place in a separate, quiet room where no other activity is taking place, and where temperature fluctuations are minimal. Clinic scheduling procedures establish consistent appointment times to minimize as much as possible the impact of daily blood pressure variation.

3.6.3.4 Measurement Procedures

The sitting arm blood pressure is measured two times at each eligibility clinic visit. It takes approximately 10 minutes to make two blood pressure measurements including the initial five minute rest. The blood pressure measurements are made early in the clinic visit sequence immediately following the reception and informed consent, and before blood drawing.

Once the applicant is given instructions and explanations, and the equipment has been checked, blood pressure measurement begins. The following steps must be followed precisely.

1. Measure the arm circumference to the nearest 1/2 centimeter.

2. Seat the applicant with the right arm on table. The bend at the elbow (cubital fossa) should be at heart level. Legs should be uncrossed and feet comfortably flat on the floor, not dangling. Be sure that the chair head support is comfortable and the participant is able to relax the neck and shoulder muscles as much as possible. Record pulse rate (number of beats in 30 seconds x 2) and record.

3. Palpate the brachial artery (just medial to and above the cubital fossa), and mark this location for stethoscope placement. Choose the correct cuff size and wrap the cuff on the arm with the center of the bladder over the artery. If the applicant seems particularly apprehensive, delay wrapping the cuff until after the five minute wait.

4. Check time. Allow a five minute wait before taking the blood pressure. Conversation should be limited, however, a brief explanation of the procedure can be repeated at this time if necessary.

5. After 5 minutes connect the cuff to a standard manometer and establish the pulse obliteration pressure by slowly inflating while palpating the radial artery until pulse is no longer felt. Deflate and disconnect the cuff. (Reminder: Peak inflation level = pulse obliteration + 30.)

6. Measurements 2: Have the applicant raise measurement arm for five seconds. After waiting another 30 seconds with the participant's arm on the table.

7. Using a calculator, average the two readings and record the average on the form.

3.6.3.5 Sitting Blood Pressure Form

Data from this form will be entered into the Data Management System.

DELTA ID

Apply the pre-printed DELTA ID label or write in the 5-character ID number on any forms or files.

Question 1: Date of Measurements

Record the date on which the blood pressures were taken. Enter the date as month/day/year using zeros to fill in all spaces.

Question 2: Arm Circumference

Measure the applicant's arm circumference in centimeters and record this value in the space provided.

Question 3: Cuff Size

Determine the appropriate cuff size according to the guidelines presented. Circle the letter corresponding to the size based on a range of values in centimeters that includes the value of the participant's arm circumference as recorded in question 2.

Question 4: Pulse

Obtain the applicant's 30 seconds pulse rate and record this value in the leftmost space provided. Then multiply the 30 second pulse rate by 2 and enter the result in beats per minute in the rightmost space provided. Enter the value recorded in the rightmost space in the data management system.

After applying the appropriate size blood pressure cuff, the applicant must sit quietly and remain seated without crossing his/her legs for 5 minutes before the blood pressure measurements are obtained.

Question 5: First Blood Pressure Measurements

Record the systolic value in 5a and the diastolic value in 5b for the first blood pressure measurements.

Question 6: Second Blood Pressure Measurements

Wait 30 seconds after the first blood pressure measurements before taking the second. Record the systolic value in 6a and the diastolic value in 6b for the second blood pressure measurements.

Question 7: Computed Average of Blood Pressure Measurements

In the workspace provided, calculate the average of the systolic and diastolic values from the first and second blood pressure measurements. Add 5a and 6a to get the sum of the systolic values and divide the sum by 2 for the average systolic blood pressure. Enter the average systolic blood pressure value in 7a. Then, add 5b and 6b to get the sum of the diastolic values and divide the sum by 2 for the average diastolic blood pressure. Enter the average diastolic blood pressure value in 7b.

Question 8: Personnel Code Number

Record the 2-digit personnel code number assigned to the DELTA study staff for the person completing this form.

DELTA ID: _____

SITTING BLOOD PRESSURE

[Measure the applicant's arm circumference and choose the appropriate cuff. After applying the cuff, the applicant must be quiet and remain continuously seated without legs crossed for 5 minutes before the two measurements. Wait 30 seconds after the 1st reading before taking the 2nd reading. Reminder: Peak inflation level = pulse obliteration + 30.]

1. Date of measurements: _____
   (mm/dd/yy)

2. Arm circumference (cm): _____

3. Cuff Size: [Circle the letter by your selection]
   P...Pediatric (<24.5 cm)
   R...Regular adult (24.5-33 cm)
   L...Large adult (33-40 cm)
   X...X-large (>40 cm)

4. Pulse: beats in 30 seconds _____ x 2 = _____ beats/minute

5. First blood pressure measurement: a. Systolic: _____ b. Diastolic: _____

6. Second blood pressure measurement: a. Systolic: _____ b. Diastolic: _____

   Calculation of average blood pressure:

   (add two values) _____ _____

   (divide sum by 2) _____ _____

7. Computed average of first and second blood pressure measurements:

   a. Systolic: _____ b. Diastolic: _____

8. Code number of person completing the form: _____

3.7 ELIGIBILITY VISIT 1 (EV1)

GENERAL REMINDERS: Please use black ball-point pen. Print all responses legibly. Initial and date all corrections. Enter data in ALL spaces provided. Enter NA or ND where applicable. (see GENERAL INSTRUCTIONS SECTION for complete instructions)

Enter the applicant's ID number, your code number, and today's date at top of page.

Part I. Self-administered Questionnaire

Please be sure the applicant reads and understands the screening consent form. They must sign the consent form before completing this questionnaire!

Give the applicant brief instructions on how to complete the form, e.g. use a black ball-point pen, print legibly, and note all questions they are not certain how to complete. After the applicant has completed the form to the best of their ability, review the answers quickly for any omissions or questions they may have. Use the following instructions to assist you with each question.

Questions 1-4: General Information

The applicant is to complete the date, their name, and birth date and emergency information. If the TSF is available, check to see that the birth date is in agreement. Also check that the applicant has completed all information requested. Ask for spelling or clarification if necessary.

Questions 5-6:

The applicant is to select a response and note the corresponding letter in the space provided.

Questions 7-8:

The applicant is to select Y for yes and N for no, and note their response in the space provided. For question 8, if the applicant has responded yes, be sure they include a description of their usual travel schedule. Remind them that the study requires participants to consume two meals, 5 days a week at the center.

Question 9:

The applicant is to read and respond to each of the foods listed by circling Y for yes, N for no and U for unsure. If they are not allergic or sensitive to any foods, they should circle N under k. "None".

Questions 10-12: Alcohol consumption

This study requires that participants consume no more than 5 alcoholic beverages a week. This series of questions is designed to address this issue. Be sure the applicant understands the definition of one drink as it relates to this question.

Questions 10-11 ask for a total number of beverages.

For example, if the applicant consumes 4, 12 oz. cans of beer and one, 1 oz. shot of liquor they should record 5 as their response.

However, if they consume 4, 16 oz. cans of beer and one, 1 oz. shot of liquor they should record 6 as their response (4, 16 oz cans is approximately equal to 5, 12 oz. cans).

For question 12, the applicant should respond Y for yes or N for no. There is **NO** unsure (U) response for this question!

Questions 13-16: Dietary Habits

The applicant is to answer Y for yes and N for no in the space provided, there is no unsure (U) response for these questions.

If the applicant responds positively to question 16, be sure they include an adequate description of their self-prescribed diet. Ask for clarification if necessary.

Question 17: Weight History

The applicant is to respond with a Y for yes or an N for no in the space provided. There is no unsure (U) response for this question.

Questions 18-21: Smoking and Exercise Habits

Question 18, parts a. and b. should be answered with a Y for yes or an N for no.

If the applicant responds with an N to 18. a. they can proceed to question 20.

If the applicant responds with an N to 18. b. they can proceed to question 19.

Question 18, part c. requests the total number of cigarettes smoked per day not the number of packs per day.

If inappropriate, the applicant may leave question 19 blank, if so, confirm this response and insert NA for not applicable.

Question 20. a. and 21. should be completed with a Y for yes or an N for no. If the applicant responds positively to question 20.a., be sure they specify what type of exercise they perform, how often, and for how many hours under 20. b-c.

Part II. Clinic Data Form

This section of the EV1 Form is to be completed by clinic personnel. **Be sure you have entered the applicant's ID number, your code number, and today's date on page 1 of this form.**

Question 22: Medical Exclusions

Read each of the medical conditions listed to the applicant and circle Y for yes, N for no **or** never tested, or U for unsure. Since this information is used for exclusion purposes, please attempt to obtain as accurate information as possible.

Question 23: Thyroid Disease

Thyroid disease is difficult to diagnose. Please attempt to obtain as accurate information as possible. Include a concise description of the nature and treatment of the disease.

Questions 24-28: Medication Use

The applicant was instructed to bring his/her medications with them to this visit. At this point in the interview, ask to see all of the medications the applicant is currently taking on a regular basis.

Enter the name of the medications prescribed by a physician, using correct spelling, under 24. b-e.

Enter the name of the medications self-prescribed, using correct spelling, under 25. b-e.

Question 26 should be answered with a Y for yes or an N for no in the space provided. There is no unsure (U) response for this question.

Questions 27-28 should be answered with a D for daily, W for weekly, O for occasionally (meaning less often than weekly), or N for never.

Questions 29-32: Body Mass Index (BMI)

Enter the applicant's height in feet and inches or meters in the space provided in question 29. Height should be without shoes rounded down to the nearest inch or centimeter.

Enter applicant's weight in pounds or kilograms in the space provided in question 30. Find the applicant's height in the BMI table provided. Note the upper weight limit listed underneath and record this value in the space provided in question 31.

If the applicant's weight in question 30 exceeds the weight recorded in question 31, enter Y for yes. If the applicant's weight does not exceed this value, enter N for no.

Questions 33-40: Sitting Blood Pressure

Measure the applicant's arm circumference in centimeters and record this value in the space provided for question 33. According to the guideline's given in question 34, select the appropriate cuff size and enter the letter corresponding to this value,e.g. if a applicant's arm is 31 cm enter R for regular adult size.

Obtain the applicant's 30 second pulse, record this value in the space indicated in question 35. Multiply the applicant's 30 second pulse by 2 and enter this value in the space indicated in question 35.

Obtain the pulse obliteration and record this value in the space indicated in question 36. Add 30 to the pulse obliteration and enter this value in the space indicated in question 36.

After applying the blood pressure cuff, the applicant must sit quietly and remain seated without their legs crossed for 5 minutes before the blood pressure measurements are obtained.

Record both the systolic and diastolic values for the first blood pressure measurement in the appropriate spaces provided in question 37.

Wait 30 seconds after the first blood pressure reading before taking the second reading. Record both systolic and diastolic values in the appropriate spaces provided in question 38.

Calculate the average systolic and diastolic blood pressure as indicated in the example below and record these values in the appropriate space indicated in question 39.

Sample calculations:

37. First blood pressure measurement	a. Systolic:	112	b. Diastolic	64
38. Second blood pressure measurement	a. Systolic:	109	b. Diastolic	62
	Total=	221		126
	Divide by 2=	110		63

According to this sample calculation, the average values to record in question 39 are 110 for systolic and 63 for diastolic.

If the applicant's average systolic blood pressure exceeds 140 **or** their average diastolic blood pressure exceeds 90, enter a Y for yes in question 40. Using the sample calculations above, you would record a N for no since the average blood pressure does not exceed these values.

Question 41: Rapid Cholesterol Screen

Obtain a rapid cholesterol check on the applicant and record the value (mg/dl) in the space provided in question 41. To remain eligible for the study, the applicant's cholesterol level must fall within the normal range for their age, race and gender as indicated in the table provided below.

Questions 42-45: For Women Only

For question 42, enter Y for yes or N for no. Enter Y only if applicant is taking female hormones as an oral contraceptive, if they are taking them for symptoms of menopause enter N and refer to question 45.

For question 43. a., indicate the applicant's current menstrual status as R for Regular, I for Irregular, or N for none at all.

If the applicant responds "none at all", complete question 43.b. by indicating the letter corresponding to the response given. Note that "Other (Specify:)" is an option for this question.

For question 44, read the question and the list of responses to the applicant. Record the letter corresponding to the response given in the space provided,

For question 45. a., enter a Y for yes if the applicant is taking any female hormones for hot flashes or symptoms of menopause. If hormones are taken as an oral contraceptive, this should be indicated in question 42. If yes, indicate when hormone therapy began in question 45.b.

Questions 46-52: Visit Checklist

Respond to each question with a Y for yes or an N for no. The answer to questions 46-50 MUST be Y for you to proceed.

Question 51 refers to food record keeping, if food records are not being kept at your center, indicate NA for not applicable.

For question 52, if the applicant is no longer eligible for the study indicate N and refer back to question 12 of the TSF. Record a Y for question 12. a. and a "B". for 12.b. indicating the applicant became ineligible at EV 1. If the applicant remains eligible, indicate Y and proceed to question 53.

Question 53: Administrative Information

Schedule an appointment with the applicant for eligibility visit 2 (EV 2) and enter the date and time in space provided for question 53.

FOR OFFICE USE ONLY

DELTA ID: _____ 1. Today's Date: _____ (mm/dd/yy) 2. Personnel Code Number: ____

Medical review needed?	**YES**	**NO**
Medical review done?	**YES**	**NO**
Eligible?	**YES**	**NO**

Welcome to the DELTA Study! We want to thank you for your help and hope that you are one of the lucky applicants who become eligible for the study. It's people like you who make research studies possible that benefit the public and answer important public health questions.

Part I (Questions 3 - 23) - To be completed by applicant

Make sure you have completed the consent screening form

3. _____
a. First Name b. MI c. Last Name

4. Date of Birth (mm/dd/yy): _____

5. Contact in case of an emergency:

a. Name: _____

b. Address: _____

c. Town/City: _____ d. State: _____ e. Zip Code: _____

f. Home telephone: _____ g. Work Telephone: _____
area-###-#### area-###-####

6. What is your highest level of schooling achieved? [Please circle the letter by your selection.]

A Eighth grade or less
B Trade school or business school instead of high school
C Some high school
D High school graduate
E Trade school or business school after graduating from high school
F Some college including 2-year degree
G Received bachelor's degree
H Graduate or professional education beyond the bachelor's degree
I Graduate or professional degree

7. a. What is your current employment status? [Please circle the letter by your selection.]

A Working a full-time job
B Working a part-time job
C Full-time or part-time student, not working
D Student working full-time or part-time
E Homemaker/Volunteer
F Retired
G Unemployed
H Disabled
I Other (b. describe: _____)

8. Do you plan to remain in the area for the next 10 months? YES NO

9. Do you have any allergies or sensitivities to any of the following foods?
[Read each of the following foods and circle your response]
YES (Y), NO (N), or UNSURE (U)

a. Meat, fish, poultry	Y	N	U
b. Shellfish	Y	N	U
c. Milk or dairy products	Y	N	U
d. If YES to milk or dairy products, is this a milk allergy?	Y	N	U
e. If YES to milk or dairy products, is this a lactose intolerance?	Y	N	U
f. Eggs	Y	N	U
g. Fruit	Y	N	U
h. Vegetables	Y	N	U
i. Nuts	Y	N	U
j. Chocolate	Y	N	U
k. Other foods (l. describe: _____)	Y	N	U

We would like to ask you a few questions about your alcohol consumption...

DEFINITION: 1 drink = a 5 oz. glass of wine, a 12 oz. can of beer, or a shot glass of liquor

10. What is the total number of alcoholic drinks that you drink Monday through Thursday?____

11. What is the maximum number of alcoholic drinks that you usually drink in any one day Monday through Thursday? ____

12. What is the total number of alcoholic drinks that you drink Friday, Saturday, and Sunday?____

13. What is the maximum number of alcoholic drinks that you usually drink in any one day Friday, Saturday, and Sunday? ____

14. Would you be willing to limit your intake to 5 drinks per week during the duration of the study? [Circle your response] YES NO

15. Are you taking any vitamins, minerals or other nutritional supplements? [An interviewer will ask you to list any nutritional supplements in Part II.] YES NO

16. Because some nutritional supplements may interfere with study results, would you be willing to stop taking this supplement if you qualify for this study? YES NO

17. Are you currently on any of the following special diets ***prescribed by a doctor*** for a medical condition? [For each special diet listed below, circle YES or NO]

a. Weight loss	YES	NO
b. Low salt or low sodium	YES	NO
c. Diabetic	YES	NO
d. Heart disease	YES	NO
e. Lower blood pressure	YES	NO
f. Weight gain	YES	NO
g. Vegetarian	YES	NO
h. Renal disease	YES	NO
i. Allergy	YES	NO
j. Other	YES	NO

(k. describe: _____)

18. a. Are you on a self-prescribed diet? YES NO

b. If YES, describe the self-prescribed diet: _____

19. Have you lost or gained more than 10 pounds within the past two months? YES NO

20. a. Have you ever smoked cigarettes? YES NO

b. If YES to 20a, do you now smoke cigarettes? YES NO

c. If YES to 20b, on average, how many cigarettes do you smoke per day? _____

21. If you have quit smoking, how many years has it been since your last cigarette? [Please circle the letter by your selection.]

A Less than 1 year
B 1 year or more

22. a. Do you exercise or play sports regularly? YES NO

If YES, please describe:

Activity	Number of Hours Per Week
b. _____	c. _____
d. _____	e. _____
f. _____	g. _____
h. _____	i. _____

23. Does your job require heavy physical labor? YES NO

End of Part I

Please hand your form to your clinic interviewer to initiate the remainder of the clinic visit.

Part II - Clinic Data Form

24. Because certain medical conditions will interfere with our study, we need to ask the following questions. Do you have any of the following medical conditions? [Read list of medical conditions and circle response YES (Y), NO (N) if NO or NEVER TESTED, or UNSURE (U)]

a.	heart disease	Y	N	U
b.	diabetes	Y	N	U
c.	high blood pressure or hypertension	Y	N	U
d.	renal or kidney disease	Y	N	U
e.	gastrointestinal condition (Crohn's disease, irritable bowel syndrome, ulcer problems, bowel surgery)	Y	N	U
f.	history of blood clotting disorders	Y	N	U
g.	liver disease (cirrhosis)	Y	N	U
h.	condition that requires steroid medication	Y	N	U
i.	gout requiring treatment	Y	N	U
j.	recent history of depression or mental illness requiring medication within last 6 months	Y	N	U
k.	anemia	Y	N	U
l.	sickle cell anemia	Y	N	U
m.	lung disease, chronic bronchitis, emphysema	Y	N	U
n.	acquired immune deficiency syndrome (AIDS) or positive HIV test	Y	N	U
o.	cancer (active within 5 years)	Y	N	U

25. a. Do you have thyroid disease or a thyroid problem? YES NO UNSURE
b. Have you ever had treatment, such as radioactive iodine or surgery, for a thyroid problem? YES NO UNSURE
c. Are you taking any medication for your thyroid? YES NO UNSURE
[If UNSURE, check the medications]

[If any medical condition was circled YES or UNSURE, then review by medical personnel is required to exclude applicant from participation.]

Medical reviewer _____ **Exclude** _____ **Include**
Initial **Initial**

26. a. Are there any medical reasons that would keep you from participating? YES NO

b. If YES, describe:

_____

27. a. Are there any personal reasons that would keep you from participating? YES NO

b. If YES, describe:

_____

28. a. Are there any professional reasons that would keep you from participating? YES NO

b. If YES, describe:

_____

WOMEN ONLY

29. a. Are you currently taking an oral contraceptive? YES NO

b. If YES to 29a, are you planning to stop? YES NO

c. If NO to 29a, are you planning to start? YES NO

30. What is your current menstrual status? [Circle the letter by your selection.]

R Regular (normal)
I Irregular
N None

31. a. If you are menstruating irregularly, what is the reason? [Circle the letter by your selection.]

A Undergoing menopause
B Other (b. describe: _____)

c. If you have stopped menstruating, what is the reason? [Circle the letter by your selection.]

A Natural menopause
B Hysterectomy
C Medication stopped period
D Other (d. describe: _____)

32. When did you have your last period? [Circle the letter by your selection.]

A Less than 2 months ago
B 2 months to 6 months ago
C More than 6 months to 1 year ago
D More than 1 year but less than 3 years ago
E At least 3 years ago

WOMEN ONLY (continued)

33. a. Are you taking or have you ever taken estrogen? [Estrogen or female hormones for hot flashes or symptoms of menopause] YES NO

b. If YES to 33a, are you currently taking estrogen? YES NO

c. If NO to 33a, do you plan to start taking estrogen? YES NO

[Resume asking questions of all applicants.]

34. How often do you take antacids? [Circle the letter by your selection.]

D Daily
W Weekly
O Occasionally
N Never

35. How often do you take laxatives? [Circle the letter by your selection.]

D Daily
W Weekly
O Occasionally
N Never

36. Within the past six months, have you taken any medications on a regular basis **prescribed by a doctor**? YES NO

If YES, specify doctor-prescribed medications, one per line: [Enter names of medications with correct spellings.]

	a. Medication Taking Medication	b. Reason for Stopped	c. Date When Plan To Resume (mm/dd/yy)	d.
37.	_____	_____	_____	YES NO
38.	_____	_____	_____	YES NO
39.	_____	_____	_____	YES NO
40.	_____	_____	_____	YES NO
41.	_____	_____	_____	YES NO
42.	_____	_____	_____	YES NO

[Applicant's doctor-prescribed medications must be confirmed at this time.]

43. a. Within the past six months, have you taken any **self-prescribed** medication or nutritional supplements on a regular basis? YES NO

If YES, please list self-prescribed medications or supplements, one per line: [Enter names of medications with correct spellings.]

b. _____

c. _____

d. _____

e. _____

f. _____

g. _____

44. If you are taking self-prescribed medications or supplements, would you be willing to discontinue use of the self-prescribed medication for the duration of this study? YES NO

HEIGHT AND WEIGHT

[Choose whether you will enter height/weight in customary units (ft-in/lb) or metric units (cm/kg) and proceed to the questions following the appropriate Upper Weight Limit Table. Only enter responses for either questions 45-47 or 48-50.]

Upper Weight Limit Table in Customary Units (ft-in/lb)

Ht.	4'10"	4'11"	5'0"	5'1"	5'2"	5'3"	5'4"	5'5"	5'6"	5'7"
Wt.	153	158	164	169	175	181	186	192	198	204

Ht.	5'8"	5'9"	5'10"	5'11"	6'0"	6'1"	6'2"	6'3"	6'4"
Wt.	210	217	223	229	236	243	249	256	263

45. Height: [Without shoes] a. ft: _____ b. in: _____

46. a. Weight: [Without shoes] lbs: _____
b. Is the applicant's weight recorded in question #46a greater than the upper weight limit for the applicant's height in the table above? YES NO

47. It is important that our participants not lose or gain weight in this study. Are you willing to participate in a study where your weight is maintained at the same level it is now? YES NO

[Proceed with instructions at the bottom of the page.]

Upper Weight Limit Table in Metric Units (cm/kg)

Ht.	148	15	152	15	156	15	16	162	16	166	168	170
Wt.	70	72	74	76	78	80	82	84	86	88	90	92

Ht.	17	17	17	178	180	182	184	186	188	190	19	194
Wt.	95	97	99	101	104	106	108	111	113	116	11	120

48. Height: [Without shoes] cm: _____

49. a. Weight: [Without shoes] kg: _____
b. Is the applicant's weight recorded in question #49a greater than the upper weight limit for the applicant's height in the table above? YES NO

50. It is important that our participants not lose or gain weight in this study. Are you willing to participate in a study where your weight is maintained at the same level it is now? YES NO

[Proceed with instructions at the bottom of the page.]

[If the applicant's weight is greater than the upper weight limit, or the applicant is not willing to maintain the same weight during the study, then the applicant has become ineligible. If so, terminate the interview.]

SITTING BLOOD PRESSURE

[Measure the applicant's arm circumference and choose the appropriate cuff. After applying the cuff, the applicant must be quiet and remain continuously seated without legs crossed for 5 minutes before the two measurements. Wait 30 seconds after the 1st reading before taking the 2nd reading. Reminder: Peak inflation level = pulse obliteration + 30.]

51. Arm circumference (cm): _____

52. Cuff Size: [Circle the letter by your selection.]

    P Pediatric (<24.5 cm)
    R Regular adult (24.5-33 cm)
    L Large adult (33-40 cm)
    X X-large (>40 cm)

53. Pulse: beats in 30 seconds _____ x 2 = _____ beats/minute

54. First blood pressure measurement: a. Systolic: _____ b. Diastolic: _____

55. Second blood pressure measurement: a. Systolic: _____ b. Diastolic: _____

56. Computed average of first and second blood pressure measurements:

    a. Systolic: _____ b. Diastolic: _____

57. Is average systolic blood pressure >140 or average diastolic pressure >90? YES NO

RAPID SCREEN CHOLESTEROL CHECK

Cholesterol Cutpoints by Gender, Race, and Age

Eligible Ranges for Total Cholesterol (mg/dl)				
Age	White Men	Black Men	White Women	Black Women
20 - 24 years	157 - 231	149 - 223	See table below for ranges for women age 20-44 years	
25 - 34 years	169 - 248	162 - 245		
35 - 44 years	188 - 273	180 - 277		
45 - 54 years	198 - 286	189 - 293	198 - 288	199 - 302
55 - 64 years	199 - 289	189 - 293	209 - 303	208 - 315
65 - 74 years	191 - 281	180 - 277	217 - 314	214 - 318

Eligible Ranges for Total Cholesterol (mg/dl) Women Age 20 - 44				
Age	White Women		Black Women	
	Oral Contraceptive User	Non-user	Oral Contraceptive User	Non-user
20 - 29 years	167 - 238	155 - 227	168 - 238	156 - 227
30 - 44 years	187 - 266	175 - 255	189 - 275	176 - 264

58. Total cholesterol level (mg/dl): _____

59. Is cholesterol level within the eligible range for the applicant's gender, race, and age?
YES
NO

[If the applicant's cholesterol level is not within the eligible range, then the applicant has become ineligible. If so, terminate the interview.]

VISIT CHECKLIST

[The following questions should all be answered 'YES' before the applicant is scheduled for Eligibility Visit 2.]

Did the applicant read and sign the consent screening form?

Was Part I of Eligibility Visit 1 completed?

Was applicant given the DELTA Information Packet?

Was the DELTA Study explained and questions addressed?

Were applicant's doctor-prescribed medications confirmed?

Does applicant remain eligible for Eligibility Visit 2?

ADMINISTRATIVE INFORMATION

60. a. Date scheduled for Eligibility Visit 2 (mm/dd/yy): _____

b. Time scheduled for Eligibility Visit 2 (hh:mm): _____ c. AM PM

[Remind applicant to come back fasting at least 8 hours to Eligibility Visit 2.]

PENNSYLVANIA STATE UNIVERSITY
DELTA STUDY

Participant Agreement

I understand that if I agree to be a participant in Dietary Effects on Lipoproteins and Thrombogenic Activity (**DELTA**), I will be expected to do the following:

1. Come to the Mateer Room for my lunch and dinner, Monday through Friday. The study will last 8 months, and I will eat 3 different diets lasting 8 weeks each during that time. Between diet periods 1 and 2, I will have a 7 week break where I can eat any food and during which I do not need to come to the study facility. Between diet periods 2 and 3, I will have a 4 week break where I can eat any food and during which I do not need to come to the study facility.

2. Pick up prepackaged containers of one meal and a snack for daily weekday use, as well as prepackaged meals for use during weekends.

3. Eat <u>all</u> foods provided. Eat <u>only</u> foods provided.

4. Weigh in at the Feeding Center to allow adjustment of calories to maintain my current body weight.

5. Avoid or limit use of all alcoholic beverages during each 8 week diet period.

6. Allow blood samples to be drawn 4 times during each diet period.

My signature indicates that I have read and understand the above description of my responsibilities in the **DELTA** study.

_____

Prospective Participant

COLUMBIA UNIVERSITY
DELTA STUDY

Participant Agreement

I understand that if I agree to be a participant in Dietary Effects on Lipoproteins and Thrombogenic Activity (**DELTA**), I will be expected to do the following:

1. Come to Bard Hall (B1) - 60 Haven Avenue for my lunch and dinner, Monday through Friday. The study will last 8 months, and I will eat 3 different diets lasting 8 weeks each during that time. Between diet periods 1 and 2, I will have a 7 week break where I can eat any food and during which I do not need to come to the study facility. Between diet periods 2 and 3, I will have a 4 week break where I can eat any food and during which I do not need to come to the study facility.

2. Pick up prepackaged containers of one meal and a snack for daily weekday consumption, as well as prepackaged meals for my consumption during weekends.

3. Eat all foods provided. Eat only foods provided.

4. Weigh in at the Feeding Center twice weekly (Monday and Thursday - before dinner) to allow adjustment of calories to maintain my current body weight.

5. Avoid use of alcoholic beverages during each 8 week diet period.

6. Allow blood samples to be drawn 4 times during each diet period.

My signature indicates that I have read and that I understand the above description of my responsibilities in the **DELTA** study.

_____

Prospective Participant

THE UNIVERSITY OF MINNESOTA
DELTA STUDY

Participant Agreement

I understand that if I agree to participate in Dietary Effect of Lipoprotein and Thrombogenic Activity (DELTA), I will be expected to do the following:

1) Come to the Moos Tower Feeding Center for breakfast and dinner, Monday through Friday. The study will include 3 diet periods of eight weeks each. I will eat 3 different diets during that time. After each 8 week diet period, I will have a break of several weeks where I can eat any food and during which I do not need to come to the study facility.

2) Pick up prepackaged containers of lunch and snacks for weekday use, as well as prepackaged meals for use during weekends.

3) Avoid all foods other than those provided or allowed by the Feeding Center and eat all foods provided by the Center.

4) Weigh in at the Feeding Center to allow adjustment of calories to maintain my current body weight.

5) Avoid or limit use of all alcoholic beverages to no more than 5 per week during each 8 week diet period.

6) Allow blood samples to be drawn 4 times during each diet period.

I understand that I may be dismissed from the study and will not receive the stated monetary compensation if I become unwilling or unable to comply with the conditions stated above.

My signature that indicates that I have read and understand the above description of my responsibilities in the DELTA Study.

Signed_____Date_____

PENNINGTON BIOMEDICAL RESEARCH CENTER
DELTA STUDY

Participant Agreement

I understand that if I agree to participate in Dietary Effect of Lipoprotein and Thrombogenic Activity (DELTA), I will be expected to do the following:

1) Come to the Pennington Center for two meals each day, Monday through Friday. The study will last 8 months, and I will eat 3 different diets lasting 8 weeks each during that time. After each 8 week diet period, I will have a break where I can eat any food and during which I do not need to come to the study facility.

2) Pick up prepackaged containers of lunch and snacks for weekday use, as well as prepackaged meals for use during weekends.

3) Avoid all foods other than those provided or allowed by the Feeding Center and eat all foods provided by the Center.

4) Weigh in at the Feeding Center to allow adjustment of calories to maintain my current body weight.

5) Limit use of all alcoholic beverages to no more than 5 per week during each 8 week diet period.

6) Allow blood samples to be drawn 4 times during each diet period.

7) I understand that I may be dismissed from the study and will not receive the stated monetary compensation if I become unwilling or unable to comply with the conditions stated above.

My signature that indicates that I have read and understand the above description of my responsibilities in the DELTA Study.

Signed_____Date_____

3.8 ELIGIBILITY VISIT 2 (EV2)

3.8.1 Purpose

1. To answer additional questions the applicant may have about the study.
2. To determine continued eligibility.
3. To measure blood pressure.
4. To measure weight and height circumferences.
5. To determine appliance availability.
6. To perform the initial fasting blood draw for baseline measurements.
7. To schedule callback to applicant to communicate eligibility for the Diet Run-In Visits, Randomization and Feeding Period following baseline blood measurements.

3.8.2 Items Needed for EV2

1. EV2 questionnaire to be completed by the interviewer.
2. Blood pressure cuff.
3. Beam balance to measure weight.
4. Instrument for measuring height.
5. Retractable inelastic metric measuring tape

3.8.3 Eligibility Visit 2 (EV2) Form

GENERAL REMINDERS: Please use black ball-point pen. Print all responses legibly. Initial and date all corrections. Enter data in ALL spaces provided. Enter NA or ND where applicable. (See GENERAL INSTRUCTIONS SECTION for complete instructions)

Enter the applicant's ID number, your code number, and today's date at top of page. Check the applicant's chart to ensure that they have signed a Participant Agreement and a Consent Form. These forms must be signed prior to continuing this visit!

Questions 1-9 Sitting Blood Pressure

Measure the applicant's arm circumference in centimeters and record this value in the space provided for question 3.

According to the guideline's given in question 4, select the appropriate cuff size and enter the letter corresponding to this value, e.g., if a applicant's arm is 31 cm enter R for regular adult size.

Obtain the applicant's 30 second pulse, record this value in the left most space indicated in question 5. Multiply the applicant's 30 second pulse by 2 and enter this value in the right most space indicated in question 5.

After applying the blood pressure cuff, the applicant must sit quietly and remain seated without their legs crossed for 5 minutes before the blood pressure measurements are obtained.

Record both the systolic and diastolic values for the first blood pressure measurement in the appropriate spaces provided in question 6.

Wait 30 seconds after the first blood pressure reading before taking the second reading. Record both systolic and diastolic values in the appropriate spaces for the second blood pressure measurement provided in question 7.

Calculate the average systolic and diastolic blood pressure as indicated in the example below and record these values in the appropriate space indicated in question 8.

Sample calculations:

6. 1st blood pressure measurement	a. Systolic: 112	b. Diastolic 64
7. 2nd blood pressure measurement	a. Systolic: 109	b. Diastolic 62
	Total= 221	126
	Divide by 2= 110	63

According to this sample calculation, the average values to record in question 39 are 110 for systolic and 63 for diastolic.

If the applicant's average systolic blood pressure exceeds 140 **or** their average diastolic blood pressure exceeds 90, enter a Y for yes in question 9. Using the sample calculations above, you would record a N for no since the average blood pressure does not exceed these values.

Questions 10-11: Waist & Hip Circumference

Follow the procedure in section 3.8.3.1. The measurements obtained for questions 10 and 11 should be obtained using a metric measuring tape. Measure the applicant's waist circumference (in millimeters; cm) two times and record the results of each measurement in questions 10a & 10b in the space provided.

Calculate the average reading of 10a & 10b and record the result in question 10c.

Measure the applicant's hip circumference (in millimeters; cm) two times and record the results of each measurement in questions 11a and 11b in the space provided. Calculate the average reading of 11a and 11b and record the results in question 11c.

Questions 12 - 13: Appliances Availability

Circle either YES or NO for each appliance listed in questions 12 and 13.

Questions 14 - 17: Blood Drawing

Prior to sending the applicant for the baseline blood draw, complete questions 14 - 17. If the answer to question 16 is NO, do not draw blood and reschedule the applicant for a fasting blood draw.

3.8.3.1 Procedure for Waist and Hip Circumference

A. Waist Circumference

1. The examiner will stand in front of the applicant. The applicant will stand erect with the abdomen relaxed, arms at sides and feet together. The applicant will expose the waist with underpants pulled below the waist. A retractable inelastic tape should be placed around the applicant in a horizontal plane at the level of the natural waist (the narrowest part of the torso). Specifically, this area is mid-way between the inferior border of the rib cage and the superior border of the iliac crests. In the obese it may be difficult to locate the waist narrowing. In this instance the smallest horizontal circumference is to be measured mid-way between the ribcage and iliac crest.

2. Examiner one and two must make certain the measuring tape is horizontal and that its diameter touches the entire circumference of the abdomen without compressing tissue.

3. The examiner will locate the point on the tape where the zero aligns with the other end of the loop. The measurement should be taken at the end of normal expiration. This measurement will be recorded to the nearest 0.1 cm.

Reliability:

The technical error of measurement for adolescents has been found to be 1.31 cm. (intrameasurer) and 1.56 cm. (intermeasurer). The technical error in the elderly has been found to be 0.48 cm. in men and 1.15 cm. in females.

3.8.3.2 Hip (Buttocks) Circumference

1. This is a measure of the circumference of the hips at the level of the trochanters (maximal extension of the buttocks).

2. The applicant will stand erect with arms at sides and feet together. The examiner will squat at the side of the applicant so the level of maximum extension of the buttocks can be visualized.

3. The inelastic tape is placed by the examiner around the maximal extension of the buttocks in a horizontal plane without causing tissue compression or indention. Examiner two will help in positioning the tape on the opposite side of the applicant's body.

4. The measurement will be recorded to the nearest 0.1 cm.

5. In the obese, the abdominal wall may sag and could accidently be included in this measurement. This is a potential problem with this measurement.

Reliability:

Little is known about the reliability of this measurement. A correlation of 0.99 between measurements one day apart was found in young men.

DELTA ID: _____ 1. Today's Date: _____ (mm/dd/yy) 2. Personnel Code Number: ____

Did the applicant read and sign the Participant Agreement? *YES* *NO*

Did the applicant read and sign the consent screening form? *YES* *NO*

SITTING BLOOD PRESSURE

[Measure the applicant's arm circumference and choose the appropriate cuff. After applying the cuff, the applicant must be quiet and remain continuously seated without legs crossed for 5 minutes before the two measurements. Wait 30 seconds after the 1st reading before taking the 2nd reading. Reminder: Peak inflation level = pulse obliteration + 30.]

3. Arm circumference (cm): _____

4. Cuff Size: [Circle the letter by your selection]
   - P Pediatric (<24.5 cm)
   - R Regular adult (24.5-33 cm)
   - L Large adult (33-40 cm)
   - X X-large (>40 cm)

5. Pulse: beats in 30 seconds _____ x 2 = _____ beats/minute

6. First blood pressure measurement: a. Systolic: _____ b. Diastolic: _____

7. Second blood pressure measurement: a. Systolic: _____ b. Diastolic: _____

8. Computed average of first and second blood pressure measurements:

   a. Systolic: _____ b. Diastolic: _____

9. Is average systolic blood pressure >140 or average diastolic pressure >90? YES NO

[If average systolic blood pressure is >140 or diastolic blood pressure is >90 on both Eligibility Visit 1 and Eligibility Visit 2, then the applicant has become ineligible. If so, terminate the interview.]

WAIST AND HIP CIRCUMFERENCE

[Refer to the manual of operations for instructions on measuring circumferences. Round the readings and average to the nearest whole numbers.]

		a. Reading 1	b. Reading 2	c. Average
10.	Waist circumference (cm)	_____	_____	_____
11.	Hip circumference (cm)	_____	_____	_____

APPLIANCES AVAILABILITY

12. Does the applicant have access to the following appliances at home? [For each appliance listed below, circle YES or NO]

a. refrigerator	YES	NO
b. freezer	YES	NO
c. microwave	YES	NO
d. stove/oven	YES	NO
e. toaster	YES	NO

13. Does the applicant have access to the following appliances at work? [For each appliance listed below, circle YES or NO]

a. refrigerator	YES	NO
b. freezer	YES	NO
c. microwave	YES	NO
d. stove/oven	YES	NO
e. toaster	YES	NO

[If the applicant has become ineligible, then terminate the interview.]

BLOOD DRAWING

14. When was the last time you ate or drank anything except water?

a. Time (hh:mm): _____ b. AM PM

15. Enter the current time?

a. Time (hh:mm): _____ b. AM PM

Calculation of fasting time:

(enter time from 15) _____

(enter time from 14) _____

(subtract for total fasting time) _____

16. Has applicant fasted at least 8 hours? YES NO

[If NO, do not draw blood and reschedule applicant in question 17.]

17. Has applicant been rescheduled for blood drawing? YES NO

If YES, enter scheduled date: a. Date: _____ (mm/dd/yy) b. Time: _____ (hh:mm) c. AM PM

[If the applicant remains eligible and has fasted at least 8 hours, send him/her for blood drawing.]

3.8.4 Baseline Lipid Profile

GENERAL REMINDERS: Use a black ball-point pen. Print all responses legibly. Initial and date all corrections. Enter data in all spaces provided. (See General Instructions Section for complete instructions.)

The top portion of the Baseline Lipid Profile Form is completed at the time of the baseline blood draw. The bottom portion of the form is completed after the blood sample is analyzed. After the form is completed in its entirety, give the form to the DELTA study coordinator for entry in the data entry system.

Enter the applicant's DELTA ID number, date blood drawn, and the phlebotomist 2-digit code number at the top of the page. The DELTA ID and date of blood draw must be completed since these items are key fields for inclusion of these responses in the data entry system.

For all dates, zero fill the numbers where necessary, i.e., 06 for June or 05 for the fifth day of the month.

After the form is completed in its entirety, give the form to the DELTA study coordinator for entry in the Data Management System.

NOTE: Questions 4 through 7 are not included on the DELTA Data Management System screens for data entry, so completion of these questions is optional.

Question 3: Sample ID

The sample ID period, week, and sample numbers are constants as indicated on the form for the baseline blood draw.

Question 4: Question to Applicant

Read question 4 to the applicant and record the number of hours since the applicant last ate or drank anything except water. If the applicant has not fasted at least 8 hours, do not draw the blood and send the applicant to the study coordinator to reschedule the baseline blood draw.

Questions 5-7: Venipuncture

Circle responses yes or no to indicate if there were any problems with the blood draw and sample processing. If the response is yes for any question, describe the problem in the space provided.

After completion of question 7, hold the Baseline Lipid Profile Form until the blood sample is analyzed. At the time the baseline blood sample is analyzed, complete questions 8-12.

Questions 8-12: Field Center Lipid Laboratory Use

In question 8, record the date blood sample analyzed.

In questions 9-11, record total cholesterol, triglycerides, and HDL cholesterol in units mg/dl. Enter total cholesterol and HDL cholesterol as whole numbers with up to 3 digits and triglycerides as a whole number with up to 4 digits.

For question 12, LDL cholesterol will be calculated by the Data Management System from the non-missing values in questions 9-11. If any value in items 9-11 is missing, LDL will be missing. The algorithm for LDL cholesterol is (total cholesterol - HDL cholesterol - triglycerides / 5).

DELTA ID: _____ 1. Date blood drawn: _____ (mm/dd/yy) 2. Phlebotomist ID: _____

3. Sample ID: a. Period: 0 b. Week: 0 c. Sample #: 1

Question to Participant

4. How many hours has it been since you ate or drank anything except water? _____

[If the applicant has not fasted at least 8 hours, then the fasting blood draw should be rescheduled.]

Venipuncture

5. a. Were there any venipuncture problems (6ml grey/red tube)? YES NO

b. If YES, describe: _____

6. a. Were there any centrifugation problems? YES NO

b. If YES, describe: _____

7. a. Were there any problems with aliquots (4 red cryovials)? YES NO

b. If YES, describe: _____

Field Center Lipid Laboratory Use

8. Date blood analyzed: _____ (mm/dd/yy)

9. Total Cholesterol (mg/dl): _____

10. Triglycerides (mg/dl): _____

11. HDL Cholesterol (mg/dl): _____

12. Calculated LDL Cholesterol (mg/dl)
[LDL cholesterol will be calculated by the data entry system from values in fields 9-11 above.]

3.8.5 Menstrual Calendar

This information will be used, along with hormonal analyses, to determine the phase of the menstrual cycle represented by each endpoint sample.

The calendars should be kept current during the 8 weeks of the diet trial. If you distribute them to the participants, you should plan to collect them at the final (week 8) blood draw for each participant. Alternatively, you may choose to keep the calendar in the participant's file and update it at each visit.

At the end of this 8-week trial, you should ship the calendars, along with two red top cryovials from each phlebotomy set from weeks 5,6,7 and 8 for hormonal analyses which will be done at the Central Lipid Laboratory (The Mary Imogene Bassett Hospital).

DELTA
Menstrual Calendar

Subject Number _____ Usual cycle length _____ days

*On the calendar below put an **X** in the block corresponding to the first day of your period (first day of bleeding). Check ✓ each day that bleeding continues enough to require a pad or tampon.*

SEPTEMBER						
SUN	MON	TUE	WED	THU	FRI	SAT
26	27	28	29	30		
OCTOBER						
SUN	MON	TUE	WED	THU	FRI	SAT
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28	29	30
31						
NOVEMBER						
SUN	MON	TUE	WED	THU	FRI	SAT
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18	19	20

*Note any **abnormalities** in your periods during the 8 weeks of the diet study with a ✓.*

		Period 1	2	3*
Start date	*earlier than usual*	___	___	___
	later than usual	___	___	___
Cramps	*worse than usual*	___	___	___
	not as bad as usual	___	___	___
Bleeding	*heavier than usual*	___	___	___
	lighter than usual	___	___	___
*Premenstrual Syndrome (PMS)***	*more severe than usual*	___	___	___
	less severe than usual	___	___	___

** Footnotes: See other side*

Turn in this calendar to study personnel at the last blood drawing.

Footnotes:

* *Most women will have only two periods during the diet study. However, three columns are provided since occasionally someone might have three bleeding periods within this time.*

** *PMS is a constellation of symptoms usually 7-10 days before menstruation that disappear with the start of the new cycle. Physical symptoms include bloating, breast swelling, pelvic pain, headache, ankle swelling, and bowel changes. Psychological symptoms include irritability, aggressiveness, depression, anxiety, tension and changes in libido.*

3.8.6 Run-In Energy Estimate

This form will be completed during the 3-day run-in phase of the study prior to participant randomization. Data from this form will not be entered into the data management system.

DELTA ID

Apply the pre-printed DELTA ID label or write in the 5-character ID number on any forms or files.

Question 1: Date of Weight Measurement

Record the date of the participant's weight measurement at the start of run-in. Enter the date as month/day/year using zeros to fill in all spaces.

Question 2: Participant's Name

Record the participant's first name, middle name, and last name in 2a-c, respectively.

Question 3: Weight

Record the participant's weight either in pounds (lbs) or kilograms (kg) in 3a or 3b, respectively. Weigh the participant before the dinner meal, without shoes or coats.

Questions 4-7: Day 1

Enter the day 1 date in question 4 as month/day/year using zeros to fill in all spaces. In question 5, record the calorie level based on the body weight in question 1. In question 6, circle only one letter corresponding to the correct response. In question 7, provide any comments in the space provided, writing legibly and avoiding abbreviations.

Questions 8-11: Day 2

Enter the day 2 date in question 8 as month/day/year using zeros to fill in all spaces. In question 9, record the calorie level based on the body weight in question 1 and responses to questions for day 1. In question 10, circle only one letter corresponding to the correct response. In question 11, provide any comments in the space provided, writing legibly and avoiding abbreviations.

Questions 12-15: Day 3

Enter the day 3 date in question 12 as month/day/year using zeros to fill in all spaces. In question 13, record the calorie level based on the body weight in question 1 and responses to questions for day 2. In question 14, circle only one letter corresponding to the correct response. In question 15, provide any comments in the space provided, writing legibly and avoiding abbreviations.

Run-In Energy Estimate

DELTA ID: _____

1. Date of weight measurement: _____ (mm/dd/yy)

2. a. First name: _____

   b. Middle name: _____

   c. Last name: _____

3. Weight, either in lbs or kg: a. lbs: _____ or b. kg: _____

Day 1 4. Date: _____ (mm/dd/yy) 5. Calorie level: _____

6. Circle correct response:
   - A Food was enough
   - B Food was too much
   - C Food was not enough

7. Comments: _____

Day 2 8. Date: _____ (mm/dd/yy) 9. Calorie level: _____

10. Circle correct response:
    - A Food was enough
    - B Food was too much
    - C Food was not enough

11. Comments: _____

Day 3 12. Date: _____ (mm/dd/yy) 13. Calorie level: _____

14. Circle correct response:
    - A Food was enough
    - B Food was too much
    - C Food was not enough

15. Comments: _____

CHAPTER 4

ADHERENCE

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4.0 ADHERENCE

Compliance with the study protocol and the study diet is a challenge equal to or greater than participant recruitment. In DELTA, assessment of adherence is important in interpreting results of the study. There is good reason to believe that our study population will be particularly responsive to a supportive and welcoming atmosphere at the clinic sites. Thus, personal interactions between the staff and participants will be very important in maintaining adherence.

In spite of our best efforts, some participants may drop out; and it will be important to record their reason for doing so on the Drop-out Form.

4.1 DROP-OUT

The Drop-Out Form will be completed at the time that a randomized participant drops out of the study for any reason. Data from this form will be entered into the Data Management System.

DELTA ID

Apply the pre-printed DELTA ID label or write in the 5-character ID number on any forms or files.

Question 1: Date of Last Visit

Record the date of the participant's last visit to the study site. Enter the date as month/day/year using zeros to fill in all spaces.

Question 2: Participant's Name

Record the participant's first name, middle name, and last name in 2a-c, respectively.

Question 3: Reason for Drop-Out

Circle only one letter corresponding to the reason for drop out from the study.

Question 4: Detailed Reason for Drop-Out

Describe the reason for drop-out in the space provided, writing legibly and avoiding abbreviations.

Question 5: Personnel Code Number

Record the 2-digit personnel code number assigned to the DELTA study staff for the person completing this form.

DELTA ID: _____

1. Date of last visit: _____
   (mm/dd/yy)

2. a. First name: _____

   b. Middle name: _____

   c. Last name: _____

3. Reason for drop-out (circle only one):

   A Failure to comply with protocol (missing 2 meals, eating 3 self-selected meals greater than 40% fat, exceeding alcohol limits more than 1 time in a period)

   B Serious illness or death

   C Voluntary withdrawal

4. Detailed reason or comments: _____

5. Code number of person completing this form: _____

4.2 MOTIVATORS TO COMPLIANCE

4.2.1 Perceptions of Personal Benefit

For the participant to perceive personal benefit from participation in the study, these aspects should be emphasized:

- close medical, dietary and weight surveillance,
- dietary counseling,
- free food,
- free baseline hematology panels,
- free coagulation studies,
- being part of a group of peers who are helping to advance medical knowledge,
- extended breaks between each of the feeding periods, and inclusion of an optional self-selected meal once a week.

4.2.2 Endorsement from Peers and Leaders

Consensual validation of the study itself and of any individual's participation in it may be a key component both in recruitment and adherence for the participants. The study investigators can obtain appropriate endorsement by developing and maintaining close associations with leaders in the community, with organizations and also with grass roots activists.

All the professional staff working in the study should be willing to provide endorsements of DELTA to the media or to interest groups. Conversely, all staff should be aware of the influence and authority they bring to such interviews or presentations and should guard against promoting negative perceptions or unfounded expectations concerning the benefits of DELTA.

4.2.3 Regular Feedback

Feedback is an effective reinforcer. Participants should be given regular feedback on their own adherence, and on the progress of the study in general and the importance of their role in its success.

The Coordinating Center will be producing a newsletter for staff and participants and will rely on the clinical sites both for information and for distribution.

4.3 INHIBITORS TO COMPLIANCE

4.3.1 Inhibitors

1. Inconvenient, unattractive, or uncomfortable facilities, lack of parking, having to wait in line.

2. Boring or unappetizing food.

4.3.2 Intercurrent illnesses and/or abnormal findings

The DELTA population is a healthy group of individuals. Results from the baseline blood samples will often contain information which, ethically, should be shared with the study applicant. This information is divided into two kinds: abnormal values which make the applicant ineligible, and abnormal values which should be shared with the eligible applicant after completion of the study.

Those values which make the applicant ineligible also need to be subdivided into abnormal values which are shared with the applicant by a letter which lists the abnormal values, and those abnormal values (panic values) which are so extreme as to necessitate contacting the applicant by telephone or in person and referring him/her for medical care. These are:

ANALYTE	VALUE EXCLUDING THE SUBJECT	PANIC VALUE
AST/ALT	above 2 x upper limit of nl	
Alkaline phosphatase	above 2 x upper limit of nl	
Bilirubin	above upper limit of nl	
Creatinine	above upper limit of nl	
BUN	above upper limit of nl	
TSH	above upper limit of nl	
Glucose	<60, >140 mg/dl	<40, >200 mg/dl
Hematocrit	below low limit for age/sex	below 20%
White blood cell count	below/above normal limit	
Triglycerides	>400 mg/dl	>1000 mg/dl
Total cholesterol	>90th percentile	

If total cholesterol is > 200 mg/dl, triglycerides > 200 mg/dl, and HDL cholesterol < 35 mg/dl, this information should also be shared with the applicant in this letter, though they are not exclusion criteria per se.

Values in participants after admission into the study may include total cholesterol > 200 mg/dl, triglycerides > 200 mg/dl, and HDL cholesterol < 35 mg/dl. These values should be shared with the participant at the end of the study.

During weekly monitoring visits, participants' complaints should be documented and evaluated in a manner that follows established medical and nursing practice. A too easy dismissal of symptoms may lead to non-compliance and drop-out or the overlooking of a symptom of a disease process which requires referral.

4.4 MASKING OF THE DIETS

The diet assignments will be masked to the Principal Investigators, coordinators, and laboratory technicians of each site; however, kitchen staff will know assignments so the correct food and beverages can be plated for service.

4.5 MANAGEMENT OF POOR ADHERENCE

Apart from the specifics discussed above, a number of general precepts apply:

Much of the potential for poor adherence can be avoided early on by adequate explanations of what the study entails and what the participant's role will be.

Prevention of poor adherence, as well as its management after the fact, will depend in large part on the relationships built between individual participants and staff. Your interpersonal skills will have a crucial impact on the participants' desire to return for each visit. Similarly, relationships among the participants will be a crucial factor; and strategies that foster group bonding should be explored and initiated.

Family and friends can be important allies both in preventing and turning around poor adherence. In fact, if they are not for you, they may well be against you, so pay attention to the participants' support systems.

Although you are expected to combat poor adherence energetically, be sensitive to the potential for inadvertently crossing the line into undue pressure or coercion.

4.6 PARTICIPANT WEEKLY MONITORING FORM

The participant's target weight for maintenance is usually the weight at EV2, since this is the weight that corresponds to their usual calorie balance.

Note that questions 5-10 on this form are asked at the first weekly interview concerning the participant's activity the previous week.

Data from this form will be entered into the Data Management System.

DELTA ID

Apply the pre-printed DELTA ID label or write in the 5-character ID number on any forms or files.

Question 1: Monday's Date

Enter Monday's date which is usually the first day each week that a participant is interviewed. Enter the date as month/day/year using zeros to fill in all spaces.

Question 2: Personnel Code Number

Record the 2-digit personnel code number assigned to the DELTA study staff for the person completing this form.

Questions 3-4: Weight

The participant will be interviewed twice each week, usually on Monday and Thursday, to record weight and other vital information.

3a-d. Enter the date, weight, and current calorie level from the first weekly interview, usually on Monday. In 3a, enter the date as month/day/year using zeros to fill in all spaces. In 3b or 3c, record the participant's current weight either in pounds (lbs) or kilograms (kg), respectively. Weigh the participant before the dinner meal, without shoes or coats. In 3d, record the calorie level based on the body weight in 3b or 3c. The calorie level is estimated and may vary according to the amount of unit foods eaten. The calorie level is adjusted such that the participant's weight does not vary 2.2 pounds (1 kg) or more from the target weight.

4a-d. Enter the date, weight, and current calorie level from the second weekly interview, usually on Thursday. In 4a, enter the date as month/day/year using zeros to fill in all spaces. In 4b or 4c, record the participant's current weight either in pounds (lbs) or kilograms (kg), respectively. Weigh the participant before the dinner meal, without shoes or coats. In 4d, record the calorie level based on the body weight in 4b or 4c. The calorie level is estimated and may vary according to the amount of unit foods eaten. The calorie level is adjusted such that the participant's weight does not vary 2.2 pounds (1 kg) or more from the target weight.

Question 5: Exercise (for week preceding first weekly interview)

In 5a, circle either YES or NO. If the response to 5a is NO, skip to question 6. If the response to 5a is YES, continue with 5b and circle one letter only corresponding to the correct response.

Questions 6-8: Illness (for week preceding first weekly interview)

6. Circle either YES or NO. If the response is NO, skip to question 9 to continue. If the response is YES, describe the illness in the space provided, writing legibly and avoiding abbreviations, and then complete questions 7 and 8.

7. In 7a, circle either YES or NO. If the response to 7a is NO, skip to question 8. If the response to 7a is YES, specify the names of the medications in 7b, 7d, or 7f and enter the corresponding weekly amounts taken in 7c, 7e, or 7g, respectively. The entries for 7b-g are text responses, left-justified in the space provided.

8. In 8a, circle either YES or NO. If the response to 8a is NO, skip to question 9. If the response to 8a is YES, answer questions 8b-d. In 8b, circle one letter only corresponding to the correct response. In 8c and 8d, circle either YES or NO.

Question 9: Smokers Only (for week preceding first weekly interview)

In 9a, circle either YES or NO. A change in smoking habits is defined as started smoking, stopped smoking, or increased or decreased smoking by at least 50 percent. If the response to 9a is NO, skip to question 10. If the response to 9a is YES, then proceed with 9b and circle one letter only corresponding to the correct response.

Question 10: Women Only (for week preceding first weekly interview)

In 10a, circle either YES or NO. If the response to 10a is YES, then proceed with 10b and record the date that the participant began menstruating.

4.6.1 Last day of blood draw

On the day of the last blood draw of each feeding period a Participant Weekly Monitoring Form will be completed for each participant. This will ensure that DELTA has recorded the required data throughout the entire feeding period.

DELTA ID: _____ 1. Monday's Date: _____ (mm/dd/yy) 2. Personnel Code Number: ____

WEIGHT

[Participants are weighed before dinner, without shoes or coats.]

3. a. Date of first weekly weight: _____ (mm/dd/yy)

First weekly weight, either in lbs or kg: b. lbs: _____ or c. kg: _____

d. Current calorie level: _____

4. a. Date of second weekly weight: _____ (mm/dd/yy)

Second weekly weight, either in lbs or kg: b. lbs: _____ or c. kg: _____

d. Current calorie level: _____

EXERCISE

[Exercise is recorded at the first weekly visit following the weekend.]

5. a. In the past week, has your exercise level changed? YES NO

b. If YES, how has your exercise level changed: [Circle letter preceding your selection]

A...More active
B...Less active
C...No exercise

ILLNESS

6. Have you been ill in the last week? YES NO [If NO, skip to question 9]

If YES, describe illness: _____

7. a. Did you take any medications for your illness? YES NO

If YES, specify the name of the medication and amount of medication:

b. Medication: _____ c. Total weekly amount: _____

d. Medication: _____ e. Total weekly amount: _____

f. Medication: _____ g. Total weekly amount: _____

ILLNESS (continued)

8. a. Did your eating change as a result of your illness? YES NO

b. If YES to 8a, how did your illness affect your eating: [Circle letter preceding your selection]
A...Ate more
B...Ate less
C...Could not eat

c. If YES to 8a, was a diet history taken? YES NO

d. If YES to 8a, was any action taken? YES NO

SMOKERS ONLY

9. a. In the last week, have your smoking habits changed? YES NO

[A change in smoking habits is defined as started smoking, stopped smoking, or increased or decreased smoking by at least 50 percent.]

b. If YES, how have your smoking habits changed: [Circle letter preceding your selection]
A...Smoking more
B...Smoking less
C...No longer smoking
D...Started smoking

WOMEN ONLY

10. a. Did you begin menstruating during the last week? YES NO

b. If YES, what date did you begin menstruating: _____
(mm/dd/yy)

4.7 MEDICATIONS THAT CAN BE USED OCCASIONALLY

Participants will be instructed to report the use of any medications to their Study Coordinators.

Headache medications: Aspirin, Tylenol, Nsaids (Motrin, Naprosyn):

Pain medications: same list

Infections: antibiotics for short term

Sleep medications and sedatives: antihistamines, hydroxyzine, over the counter drugs

Cold and allergy medications: antihistamines with/without ephedrine or other beta adrenergies -- many of these are over the counter combinations that should be ok as long as they do not contain steroids.

Laxatives: Milk of Magnesia, senna and cascara -- only occasional use

Antidiarrheal: Lomotil, Kaopectate -- only occasional use

Cough medicines: Robitussin, Ny-Quil

CHAPTER 5

DIET

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5.1 GENERAL INSTRUCTIONS

Rigorous control of the diet is critical to the success of the DELTA study. Procedures are directed at controlling sources of variability to the maximum extent compatible with practicality and compliance. Sources of variability include procurement, preparation, (weighing, cooking) and consumption.

Fat sources and foods with a stable shelf life are procured centrally for all field centers. Skim milk is procured locally from a dairy that can guarantee skim milk with a fat content $<0.5\%$. Whole milk is also procured locally and will provide 3.25 (e.g.: 3.3) % of fat. Perishable fruits and vegetables are procured locally according to standard specifications.

5.1.1 Weighing

In order to minimize variability contributed by weighing of food portions in individual centers, Nutrient Composition Laboratory (NCL) has provided identical sets of weights to each center, including the FALCC. These weights have been calibrated against an NIST weight set (Class P Certified). Please provide the simple instructions to your staff members for their daily use.

If you have any questions please call Carol Davis, NCL 301-504-8356.

First Weighing (beginning of each day):

- Wear fat-free, powder-free gloves
- Clean balance pan
- Level balance if necessary
- Zero balance
- Use gloves and forceps when handling weights:

- Weigh 1, 10, and 100 gram weights and record their weights on the calibration form (see page 5.1.2.1). For the 100g weight be sure to use both hands and forceps (one pair for each side).

5.1.2 Acceptable Ranges

Weights you obtain should fall within the following ranges for the nominal weights assigned to each. **If weight falls outside of the acceptable range repeat weighing procedure to verify, then call for service**.

The supervisor or co-worker should verify the calibration and initial the calibration form at the beginning of each day.

NOMINAL WEIGHT	TOLERANCE	2 X TOLERANCE
1 gram	.005g	.01g
10 graM	.020g	.04g
100 gram	.030g	.06g

1a. If balance is accurate to tenths and w = weight, then:

NOMINAL WEIGHT	ACCEPTABLE RANGE*	ACC/RANGE ROUNDED TO TENTHS
1 gram	0.99 - 1.01	1.0 - 1.0
10 gram	9.96 - 10.04	10.0 - 10.0
100 gram	99.94 - 100.06	99.9 - 100.1

1b. If balance is accurate to hundredths and w = weight, then:

NOMINAL WEIGHT	ACCEPTABLE RANGE*	ACC/RANGE ROUNDED TO HUNDREDTHS
1 gram	0.99 - 1.01	.99 - 1.01
10 gram	9.96 - 10.04	9.96 - 10.04
100 gram	99.94 - 100.06	99.94 - 100.06

*(The acceptable weight range is calculated by allowing two times the tolerance range.)

5.1.2.1 DELTA Calibration Form for Food Balances

Center Name: _____ Scale #: _____

Please complete the following form every morning. Use a separate sheet for each scale. These data may be needed by the Coordinating Center. If first weight is out of range, supervisor or co-worker should repeat the calibration. If second weight is out of range, service is required.

Date	1 gram Range 0.9-1.1 First Wt.	Second Wt.	10 gram Range 9.9-10.1 First Wt.	Second Wt.	100 gram Range 99.9-100.1 First Wt.	Second Wt.	Calibration Checked by (Please Initial)	Service Required (Yes/No)

5.1.3 Containers

In order to avoid losses in transferring food, it is preferable to weigh directly into the serving container. Cooked foods may be cooked and served in the same container. This is particularly important where there may be fat loss during cooking. If the food is not served in its cooking container, a rubber spatula should be used to transfer any food adhering to the cooking container onto the serving dish. Food must be cooked in non-stick cookware or cookware sprayed with "Mazola No-Stick" spray. Follow specifications in recipe.

5.1.4 Meat, Fish, Poultry

Meat, fish and poultry are stored frozen. These items should always be thawed in the refrigerator. Remove any visible fat, drain and pat dry with paper towels before weighing. Chicken should be washed and dried before weighing. Meat products packed in vacuum pack plastic or precooked products do not need washing.

5.1.5 Canned Fruits and Vegetables

Drain canned fruits and water packed vegetables in a fine mesh strainer for 5 minutes before weighing. Baked beans are weighed in their sauce.

5.1.6 Pasta and Rice

To cook rice, follow the exact directions in recipe. Let cool to room temperature and then weigh. To cook pasta, bring water to a rolling boil before adding the pasta. Cook for the exact time specified on the package and then rinse and drain immediately. Cool to room temperature before weighing. For rice cooked with other ingredients, follow instructions in the recipe.

5.1.7 Modified Procedures to Reduce Labor

Large scale feeding studies, such as DELTA-1, incur a heavy investment in labor in order to maintain rigid dietary control. This need has to be balanced against practical aspects of cost and efficiency without sacrificing the integrity of the study. After validating the experimental diets using procedures deemed to give maximum control of variability in the diets, which showed the diets to be essentially on target, some procedures have been modified to reduce food production time. These modifications involve foods that are not significant sources of fat or cholesterol. Modifications include batch preparation of food mixtures, measurement by calibrated volume measures, and weighing within a tolerance range of ± 1g. Substitution of volume for weighed measurement is optional.

Specific allowed modifications are listed in the following sections. Each center should record in its log which of the allowed modifications have been implemented in its food procedures.

The quality control assay results will be used to assess the impact of these procedure modifications on the integrity of the diet as fed to the participants. Therefore, items sampled from the production should be a true random sample of all items prepared, and not a sample specifically weighed/measured for quality control. Sampling of the foods should not be done by the same person who prepared the portion.

5.1.7.1 Food Items that can be weight ± 1 gram:

MENU 1

Breakfast	Orange Juice Bran Flakes PF Wheat Bread Applesauce
Lunch	White Bread Ginger cookie ingredients: Sugar Molasses Vinegar Flour Baking Soda Ginger, Cinnamon, Cloves
Dinner	Egg Noodles Green Peas Carrots Tomato PF Country Classic Roll Peach Slices
Snack	Raisins Pretzel Sticks

MENU 2

Breakfast	Orange Segments Raisin Bran PF White Bread
Lunch	Shrimp Pasta Salad Ingredients: Spiral Shaped pasta Green Onion Celery Broccoli Florets Cherry Tomatoes Lemon Juice

Vinegar
Spice Mix
PF Sourdough French Roll
Oatmeal Cookie Ingredients:
Light Brown Sugar
White Sugar
Flour
Baking Powder
Rolled oats

Dinner

Chicken Risotto Ingredients:
Mushrooms
White Rice
Water
Spice (Saffron)
Spinach
Green Onion
PF French Style Roll
Fruit Cocktail

MENU 3

Breakfast

Orange Juice
Cheerios
PF English Muffin

Lunch

Chicken Salad Ingredients:
Celery
Sweet Pickle Relish
Onion
Lemon Juice
Salt and Pepper
PF Wheat Bread
Tomato
Melon Sections

Dinner

Vermicelli
Peas
Carrots
Tomato
Green Pepper
PF Country Classic Roll
Rolled oat Macaroon Ingredients:
Sugar
Rolled Oats
Baking Powder
Vanilla
Salt

MENU 4

Breakfast	Orange Juice Bran Flakes PF Wheat Bread Applesauce
Lunch	PF Onion Sandwich Bun New Potato Salad Ingredients: Skinless Boiled Potatoes Green Onions Tomato Lemon Juice Peach Slices
Dinner	Turkey Almond Casserole Ingredients: Egg Noodles Spice Mix Bread Crumbs Green Beans Tomato PF Country Classic Roll Ginger Cookie Ingredients (See MENU 1)
Snack	Raisins Pretzel Sticks

MENU 5

Breakfast	Grapefruit Segments Raisin Bran PF White Bread
Lunch	Chicken Pasta Salad Ingredients: Green Pepper (instead of Red Bell Pepper) Broccoli Carrots Rotini Tomato Cider Vinegar Lemon Juice Mustard Spices Spinach Leaves PF Sourdough French Roll Fruit Cocktail

Dinner

Scallion Rice Ingredients:
- Water
- Chicken Bouillon
- Long Grain Rice
- Sliced Green Onions

Carrots
Peas
PF French Style Roll
Oatmeal Cookie Ingredients (see MENU 2)

MENU 6

Breakfast

Apple Juice
Cheerios
PF English Muffin

Lunch

Carrot
Green Peas
Garlic
Green Pepper
Rice
Rolled Oat Macaroons Ingredients (see MENU 3)

Dinner

All Seasoned bread Crumb Ingredients
Marinara Sauce Ingredients:
- Onion
- Garlic
- Tomatoes
- Spices
- Tomato Paste
- Cooking Wine

Vermicelli
Tomato
Melon Sections
PF Country Classic Roll

5.1.7.2 Salad ingredients:

Lettuce
Spinach
Green Onions
Tomatoes
Green Peppers

PORTION CONTROL CHANGES TO DDFR
September 30, 1993

1) All diets, all menus,

1500, 2000, 2500 kcal:	use: 2 t. (1 PC) (14 g) jelly
3000 & 3500 kcal:	4 t. (2 PC) jelly

2) Diet B, menu 5, all kcal: add apricot jelly

3) Diet C, menu 1, all kcal: add 1 TBS sugar to applesauce*

4) Diet C, menu 4, all kcal: add 1 TBS sugar to applesauce*

5) Diet A, menu 1, 1500 kcal: delete Dijon mustard at lunch

6) All diets, menu 4,

1500, 2000, 2500 kcal:	use: 1 t. (1 PC, 5g) Dijon mustard
3000 & 3500 kcal:	use: 2 t. (2 PC) Dijon mustard

7) All diets, lunch, menus 1 & 3

1500, 2000, 2500 kcal:	use: ¼ cup lettuce
3000 & 3500 kcal:	use: ½ cup lettuce

8) All diets, dinner, menus 1, 3, 4 & 6

1500, 2000, 2500 kcal:	use: ½ cup lettuce in tossed salad
3000 & 3500 kcal:	use: ¾ cup lettuce in tossed salad (item on DDFR is "1 sv")

9) Diet A, menu 6, all kcal: use: FF Italian dressing (in place of FF Ranch)

10) Diet B, menu 4, 1500 2000, 2500 kcal: use: 1 TBS FF dressing (decrease from 2 TBS)

11) All diets, menus 1, 3, 4, 3000 & 3500 kcal: use: 2 TBS FF dressing

*The sugar replaces 12 g carbohydrate from the jelly. This brings the jelly down to levels more consistent with the other menus.

CALCULATIONS USING SWEETENED APPLESAUCE

For 100g Unsweetened Applesauce - Use 56g Sweetened Applesauce

Equivalents:

Unsweetened	Sweetened
125g	70g
150g	84g
165g	92g
180g	101g
200g	112g
225g	126g
275g	154g

5.2 DIET, MENU, AND CALORIE ASSIGNMENT

During the run-in period, each participant will be fed one day of each experimental diet over a three-day period. In order to maintain masking, a different menu will be fed for each of the diets. The calorie level selected for the run-in should be on the generous side to avoid any negative reaction to the study because of hunger. The estimated calorie level is calculated from the height and weight obtained at EV1 according to the Harris Benedict formulae:

Harris-Benedict Equations (HBE) for Basal Energy Expenditure (BEE):

BEE (Females) = 655 + 9.46 Wt (kg) + 1.86 Ht (cm) - 4.68(yr)
BEE (Males) = 66.47 + 13.75 Wt (kg) + 5 Ht (cm) - 6.76 Age (yr)

TC = BEE x 1.6	Moderate activity*
TC = BEE x 1.5	Light activity
TC = BEE x 1.3	Sedentary
TC = Total Calories	

(A program has been installed in your data entry system to calculate this value at EV1).

***Note:** Current experience suggests that most participants will require a factor of at least 1.6 X BEE.

Each participant who is randomized after run-in, will be assigned a diet sequence and initial calorie level. All participants will begin the study with menu 03 at the dinner meal. This will allow the kitchen personnel time to make up production schedules. Each subsequent diet period will begin with menu 03.

DELTA MENU SCHEDULE - PROTOCOL I, FEEDING PERIOD 1

Menu Sequence
Calendar for DELTA Team
OCTOBER, 1993

SUN	MON	TUE	WED	THU	FRI	SAT
					1 1	2 W1
3 W2	4 2	5 3	6 4	7 5	8 6	9 W3
10 W4	11 1	12 2	13 3	14 4	15 5	16 W1
17 W2	18 6	19 1	20 2	21 3	22 4	23 W3
24 W4	25 5	26 6	27 1	28 2	29 3	30 W1
31 W2						

Menu Sequence
Calendar for DELTA Team
SEPTEMBER, 1993

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
			1	2	3	4
5	6	7 Labor Day	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27 3	28 4	29 5	30 6		
	Begin Per. 1					

Menu Sequence
Calendar for DELTA Team
NOVEMBER, 1993

SUN	MON	TUE	WED	THU	FRI	SAT
	1 4	2 5	3 6	4 1	5 2	6 W3
7 W4	8 3	9 4	10 5	11 6	12 1	13 W1
14 W2	15 2	16 3	17 4	18 5	19 6 *End Per. 1*	20
21	22	23	24	25 Thanksgiving	26 Holiday	27
28	29	30				

DELTA MENU SCHEDULE - PROTOCOL I, FEEDING PERIOD 2

Menu Sequence
Calendar for DELTA Team
JANUARY, 1994

SUN	MON	TUE	WED	THU	FRI	SAT
						1 *Happy New Year!*
2	3	4	5	6	7	8
9	10 3 *Begin Per. 2*	11 4	12 5	13 6	14 1	15 W1
16 W2	17 2	18 3	19 4	20 5	21 6	22 W3
23 W4	24 1	25 2	26 3	27 4	28 5	29 W1
30 W2	31 6					

Menu Sequence
Calendar for DELTA Team
FEBRUARY, 1994

SUN	MON	TUE	WED	THU	FRI	SAT
		1 1	2 2	3 3	4 4	5 W3
6 W4	7 5	8 6	9 1	10 2	11 3	12 W1
13 W2	14 4	15 5	16 6	17 1	18 2	19 W3
20 W4	21 3	22 4	23 5	24 6	25 1	26 W1
27 W2	28 2					

Menu Sequence
Calendar for DELTA Team
MARCH, 1994

SUN	MON	TUE	WED	THU	FRI	SAT
		1 3	2 4	3 5	4 6 *End Per 2*	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30	31		

DELTA MENU SCHEDULE - PROTOCOL I, FEEDING PERIOD 3

Menu Sequence
Calendar for DELTA Team
APRIL, 1994

SUN	MON	TUE	WED	THU	FRI	SAT
					1 *GOOD FRIDAY*	2
3	4 3 *Begin Per 3*	5 4	6 5	7 6	8 1	9 W3
10 W4	11 2	12 3	13 4	14 5	15 6	16 W1
17 W2	18 1	19 2	20 3	21 4	22 5	23 W3
24 W4	25 6	26 1	27 2	28 3	29 4	30 W1

Menu Sequence
Calendar for DELTA Team
MAY, 1994

SUN	MON	TUE	WED	THU	FRI	SAT
1 W2	2 5	3 6	4 1	5 2	6 3	7 W3
8 W4	9 4	10 5	11 6	12 1	13 2	14 W1
15 W2	16 3	17 4	18 5	19 6	20 1	21 W3
22 W4	23 2	24 3	25 4	26 5	27 6 *End of Study!*	28
29	30 *HOLIDAY!*	31				

5.3 FEEDING

All centers will serve two meals a day, Monday through Friday, at the feeding site. One of the meals must be dinner. Meals not eaten at the center, snacks and free beverages will be packaged in suitable containers for consumption off site (see Food Safety, section 5.8.5.1). Participants must eat and drink all study foods provided except for discretionary items. Participants should be given instruction in how to use either bread or a rubber spatula to be sure they consume all food residues. Rubber spatulas should be provided for home use.

5.3.1 Menu cycles

Weekday menus are numbered 01 through 06. Weekend menus are numbered W1 through W4. Menus will be rotated sequentially as shown in the schedule, section 5.9.4.2.

Feeding begins at dinner on September 27, with menu 03. On any given day each center is feeding the same menu. All participants will receive the same menu except in unusual circumstances (see section 5.7).

5.3.2 Unit foods

Three unit foods have been formulated in 150 kcal units that contain the same composition and the same fatty acid profile of the diets. Unit foods are used to fine tune energy adjustment (see section 5.5) and to provide extra snacks if a participant gets hungry outside of scheduled meals. Participants are expected but not required to consume all of the unit foods provided once they are stabilized at a calorie level.

5.3.3 Discretionary beverages and seasonings

Discretionary beverages:

A limited number of alcoholic and caffeinated beverages are allowed. Alcoholic beverages may be consumed up to 5 drinks per week. One drink is defined as:

1 shot (1.5 oz.) hard liquor
1 12 oz. can of beer
1 5 oz. glass of dry table wine

Cordials (liqueurs) and mixed drinks containing sugar are not permitted.
No alcoholic beverages are permitted 48 hours prior to blood drawing.

Caffeinated beverages not containing sugar may be consumed in amounts up to the following limits:

5 (6 oz.) cups coffee or tea
5 12 oz. glasses iced tea
5 12 oz. cans diet soda: Diet Coke, Diet Cherry Coke, Diet 7-UP Gold, TAB, Diet Rite Cola, Diet Dr. Pepper, Diet Cherry Cola, Diet Pepsi, Jamaica Cola, Canada Dry

Non-caffeinated diet or unsweetened beverages may be consumed in any amount.

Sugar-free chewing gum is allowed.

Discretionary seasonings:

The following seasonings are allowed for discretionary use: salt, pepper, Mrs. Dash's, Seasoned salt, Lemon pepper, Tony's (Louisiana), Tabasco or other red pepper sauce, Vinegar, Lemon juice.

The following seasonings are not allowed for discretionary use: soy sauce and Worcestershire or similar steak sauces containing fermented products and/or anchovies.

5.4 LABELS AND MASKING

Each food service facility has in place standard procedures for producing and delivering the diet and these will be maintained in the DELTA study. The critical factors the procedures should ensure are verification that the participant has received the correct diet and that masking of the participant is maintained. Masking of nutritionists is not feasible because they will have supervisory and counseling/compliance responsibilities. Nutrition personnel will be instructed 1) to maintain the same level of activity and interaction with each participant regardless of diet assignment and 2) to avoid revealing a participant's diet assignment.

Described below are the procedures each center will use to verify the diet and maintain masking of the participants.

5.4.1 Columbia

Spreadsheets are used to weigh the individual foods for each calorie and fat level. Recipes are used to prepare combination foods. Each food is labeled with the participant's name, I.D., and calorie level.

Each menu item (e.g., peaches) for each diet and calorie level are weighed and labeled with the participant's name and I.D. and placed directly in the refrigerator.

Each participant's set of labeled foods are assembled as the participant arrives for his/her meal. This system maintains masking of the experimental diets.

For packaged meals, the bags are labeled with the name of the participant and stapled with the DELTA packed meal form. The corner of the form indicating the diet assignment is cut off before the participant receives the packed meal. This form lists the food items in the bag to help the participant verify receipt and consumption of all items. The participant returns the form to the feeding site the next day.

5.4.2 Pennington Biomedical Research Center (PBRC)

Labels for meal trays and carry-out boxes are printed with the participant's name, ID number and study.

All products received are labelled with the study name and dated. As recipes are prepared, the foods are placed into appropriate storage containers, labelled with the study name, food item, and treatment. Color dots are used to identify treatments.

As meal trays are prepared, the daily diet lists for each participant are followed. The diet lists are color-coded to match color dots used for each treatment. Any identifying mark (i.e.: color dots) are removed when a food item is placed on individual trays or in carry-out boxes.

Packed meals (individual and weekend) are assembled similarly. All labels on each food are removed before the food is packed to maintain masking of the experimental diets. Participants will return the Packed Meal Forms to the feeding center.

5.4.3 Minnesota

The system of coding diets has been based on color coding. Each diet has a specific color menu and label system which includes the diet color code, study name and calorie level. A different color menu and corresponding spread sheet is used in preparation of each of the diets (i.e.: Diet A = Pink, Diet B = Blue, Diet C = Yellow). The food items will be labeled as they are prepared with the study name, color code, and caloric level. Meal trays will be assembled using the checklist on the DELTA Daily Food Record. Prepared recipes and weighed food items are stored with labels stating the study, diet color code, and calorie level. Packed meals will be appropriately labeled to allow for ease in assembly of the meal, and each meal will be separately packed using the checklist on the Packed Meal Form.

5.4.4 Penn State

For each meal we will use a form that includes the specific quantities of each menu item for each experimental diet for each calorie level. The form will also provide information about the number of participants on each experimental diet and calorie level.

Each menu item will be portioned out by weight or household measure for all experimental diets and calorie levels. We will label each one with the diet and calorie level, assemble them on a tray and put the tray in the refrigerator.

Thirty minutes before mealtime, we will start to assemble the participant's trays. The trays will have a name placard and a label with each participant's name, diet and calorie level. The individual trays for a specified diet and calorie level will be assembled at one time, followed by participants' trays for a different experimental diet and calorie level, etc., until all the trays have been assembled. We will assemble each tray using the DELTA Daily Food Record. As we place the individual food items on the tray, we will remove all the labels from the menu items and the tray. Only the placard with the participant's name will be left on the tray. The tray will be placed on a rack in the refrigerator that is labeled with the participant's name. This system will maintain masking of the experimental diets.

Packed meals will be assembled similarly. All labels on each food will be removed before the food is packed to maintain masking of the experimental diets.

5.5 ENERGY ADJUSTMENT

Adjustments in the calorie level may be made during the run-in period based on the participant's perception of whether the amount of food is too much or too little. Thereafter, adjustments will be made based on body weight monitoring. See weekly monitoring (PWM) form.

Menus are calculated at 5 standard calorie levels: 1500, 2000, 2500, 3000 and 3500. If the participant's weight varies $\geq$ 1 Kg. (2.2 lbs) from the baseline weight, the calorie level is lowered or raised accordingly. Baseline weight is defined as the first weekly weight of each feeding period. If a participant's weight has changed in the interval between feeding periods weight should be maintained at the current level. Unit foods can be used to attain a level midway between the standard calorie levels. Each unit food is 150 calories. Alternatively, if a participant is on a calorie level that is between standard calorie levels she/he will be placed on the next higher calorie level.

Weight gain in premenopausal women is evaluated in relation to the menstrual period. If weight gain occurs during the menstrual period, reduction in energy intake should be made only if the weight gain persists one week after the menstrual period.

5.6 SELF-SELECTED MEAL

Participants are allowed one self-selected meal in place of the Saturday evening meal. Participants are given guidelines to select a meal that is 30% fat. See Figure 5.6.1. Use of the self-selected meal is optional. Participants may choose to have all weekend meals provided by the field center.

Each center will use it's own procedure for ascertaining the composition of the self-selected meal. Items eaten at the self-selected meals (if used by participant) should be listed on the Packed Meal Form by the participant and then transferred to the back of Saturday's DDFR by study personnel. This information will be used only for counseling and will not be analyzed.

5.6.1 Sample Welcome Letter

Dear DELTA Participants,

Welcome to _____.

You are allowed one "self-selected" meal each weekend if you wish. This meal will be in place of one of the sandwich meals. This means that you will get a breakfast and a hot meal from the center. If you eat your self-selected meal in the evening, you will get your DELTA hot meal for lunch. We will still package all meals for the weekend so you can make last minute plans to eat out. We will ask you on _____ whether or not you will want DELTA meals for the entire weekend. Your "self-selected" meal must be similar to the study diet so we are giving you some suggestions for making good choices.

1. Choose one of the following meal plans, and eat a variety of foods from within the plan.

2. The portions of meat, poultry (without skin), fish and cheese should be limited to 3 oz. 3 oz of food is equivalent to the size of a deck of playing cards.

3. Avoid deep fried foods, cream sauces, cheese sauces, gravy and very large portions.

4. The following seasonings will be allowed for table use: Mrs. Dash's, Seasoned salt, Lemon pepper, Tony's (Louisiana), Tabasco or other red pepper sauce, Vinegar, Lemon juice. The following seasonings will not be allowed for table use: Soy sauce and Worcestershire or similar steak sauces containing fermented products and/or anchovies.

5. The amount of dressing or oil you add to your salad should also be limited to the amount listed below. Use diet dressing if you need more.

6. One serving of fresh or canned fruit is equal to 1 medium sized piece of fresh fruit or ½ cup fruit packed in its own juice.

7. Please record everything you eat or drink for the self-selected meal on the food record form provided. You may refer to the pictures of meat sizes, cup and spoons to help describe the portions of food you are eating.

8. Please complete the DELTA Packed Meal forms for your weekend meals and snacks.

Bon Appetit!

5.6.2 Guidelines for Self-selected Meal

Plan I

3 oz	hamburger--Quarter pounder "HOLD THE CHEESE" on a large bun
2 teaspoons	ketchup or mustard if desired
1-2 cups	salad--mixed green
1 Tbsp	Fat Free Italian or French dressing
1 can	coke or other soda or 10 oz orange or apple juice
1 serving	fresh or canned fruit

(You may substitute lamb, pork or veal--ALL LEAN--for the hamburger)

Plan II

1-½	cups cooked spaghetti
3	meat balls--each 1" diameter
1 - 2 cups	mixed green salad or 1 cup cooked veggies
1 Tbsp	Fat Free Italian or French dressing
1 serving	fresh or canned fruit
1 can	Coke or other soda or 10 oz orange or apple juice

Plan III

3 oz	Lean Roast Beef sandwich, bread of your choice. NO croissant.
1 - 2 cups	mixed green salad
1 Tbsp	Fat Free Russian dressing
1 serving	fresh or canned fruit
1 can	regular soda of your choice

Plan IV

2 - 3 slices	Thin 'N Crispy Pizza with veggies if desired. NO meat topping, NO double cheese pizza.
1 can	regular soda of your choice
1 large	fresh fruit

Plan V

3 oz	Turkey breast--in a sandwich or as a hot meal with 1 cup of pasta or large baked potato
½ cup, 1 scoop	standard ice cream, NO Hagen-Daz or other Premium ice cream. NO fudge, caramel, or nut sauces

1 - 2 cups	salad with 1 Tbsp Fat Free Italian dressing
1 serving	fresh fruit or canned
2 teaspoons	olive oil in pasta or 2 teaspoons mayo on the sandwich

(You may have ¾ oz (1 slice) cheese and 4 premium type crackers in place of the ½ cup ice cream.)

Plan VI CHINESE RESTAURANT

1 cup	Wonton soup only (Avoid egg-drop soup, no egg rolls)
2 cups or 2-3 cups	Moo Goo Gai Pan Chicken or Shrimp Chow Mein or Chop Suey (Avoid Egg Foo Young or any menu listing that is made with lobster sauce or in which the meat is first fried in hot oil.
2 cups	Hot steamed rice
1/2 cup	Steamed vegetables. Choose dishes that state boiled, steamed or lightly stir-fried rather than sauteed.
1 each	Fortune cookie
	Tea or soda

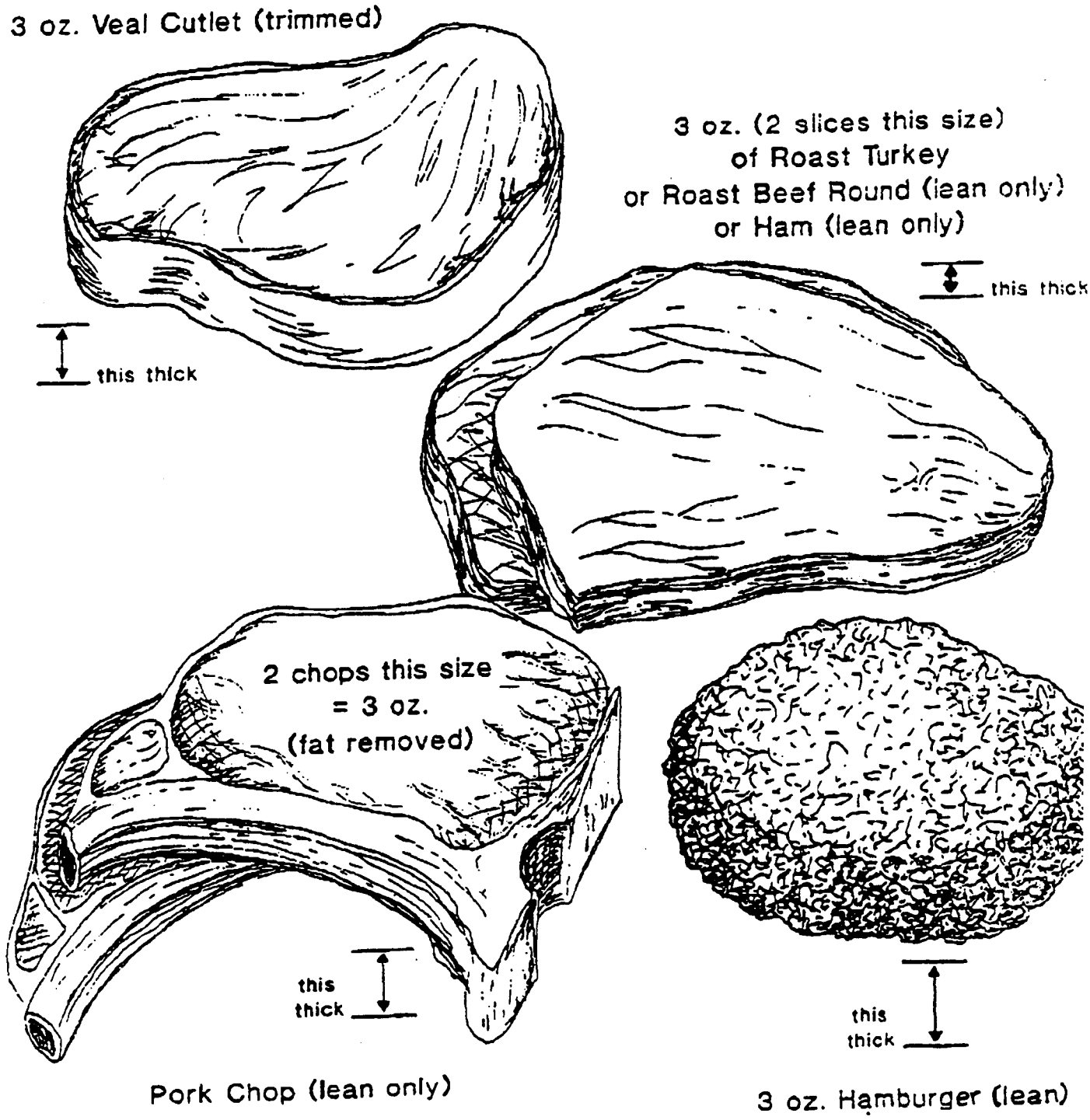
Plan VII MEXICAN RESTAURANT

1 cup	Chili Con Carne with Beans - no cheese or sour cream added
1 each or 1	Chicken Fajita* Chicken or Shrimp Tostado. Request tortillas made with cornmeal and baked rather than fried. Request garnishes,* such as cheese and sour cream, be served on the side.
1-2 cups	Salad greens, tomato, onion and avocado. Use salsa generously for dressing.
1 cup	Spanish rice. Avoid refried beans.
	No dessert other than fruit Regular soda of your choice

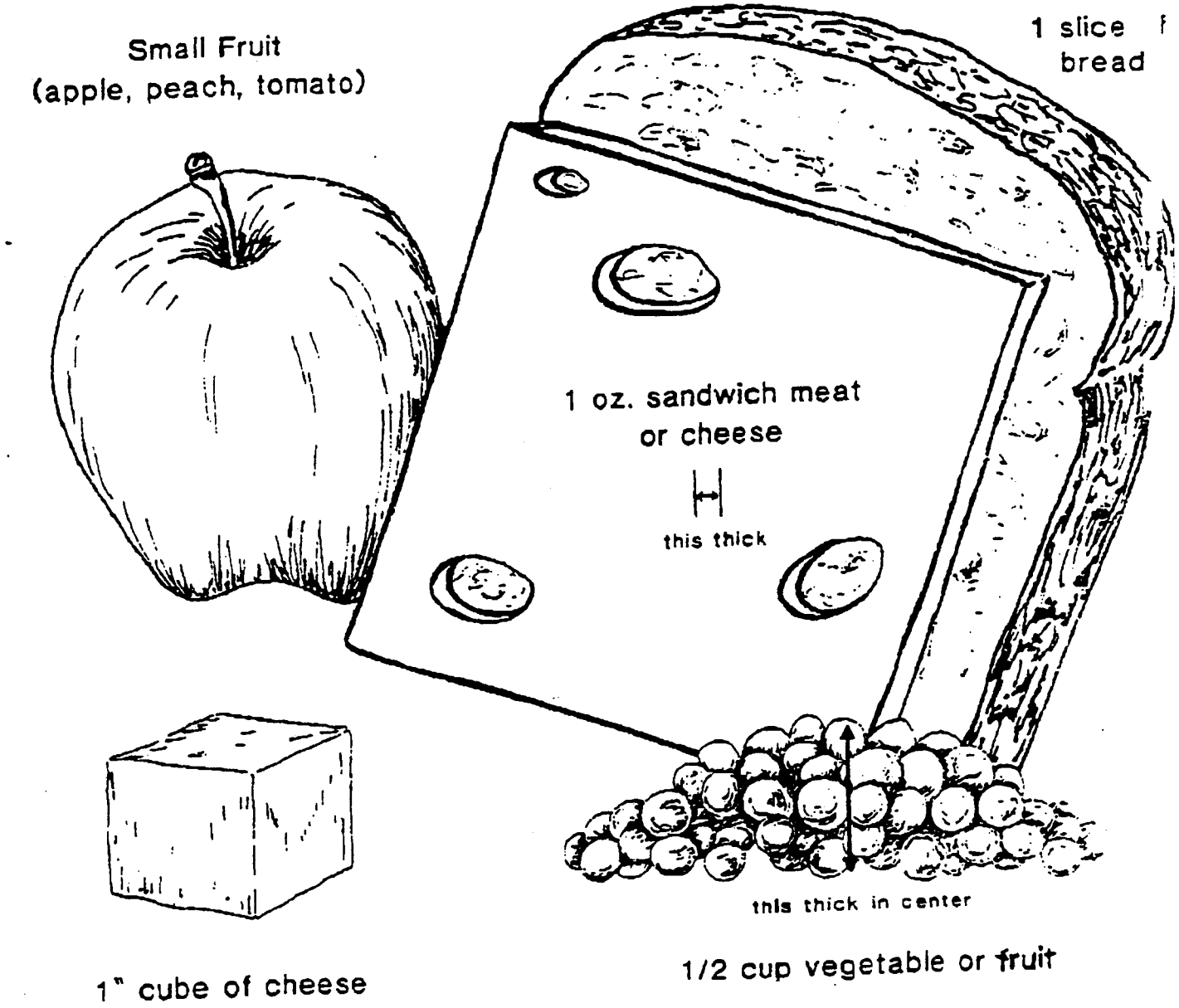
*but limit to 1 TB of cheese or sour cream

Plan VIII	STEAKHOUSE RESTAURANT
3 oz.	London broil, fillet mignon, round or flank steak broiled without added fat
1-2 cups	Mixed green salad
1T	Salad dressing on the side: Fat Free French, Italian or Thousand Island
1 large	Baked potato. NO sour cream or bacon bits.
1 pat	Margarine
1 serving	Steamed vegetables as accompaniment
1/2 cup	Sherbet
1	Roll
Plan IX	SEAFOOD RESTAURANT
3 oz. or 6 oz. or 18	Broiled fish Crabs, lobster, scallops, shrimp or clams Steamed oysters Order broiled, boiled or steamed simple dishes without additional fat. Avoid casseroles.
1/2 cup	Rice Pilaf
1 cup 1T	Lettuce salad Fat Free Russian Dressing
1/2 cup	Steamed Vegetables
1	Dinner roll
1 pat	Margarine

SERVING SIZES OF MEATS



COMMON SERVING SIZES

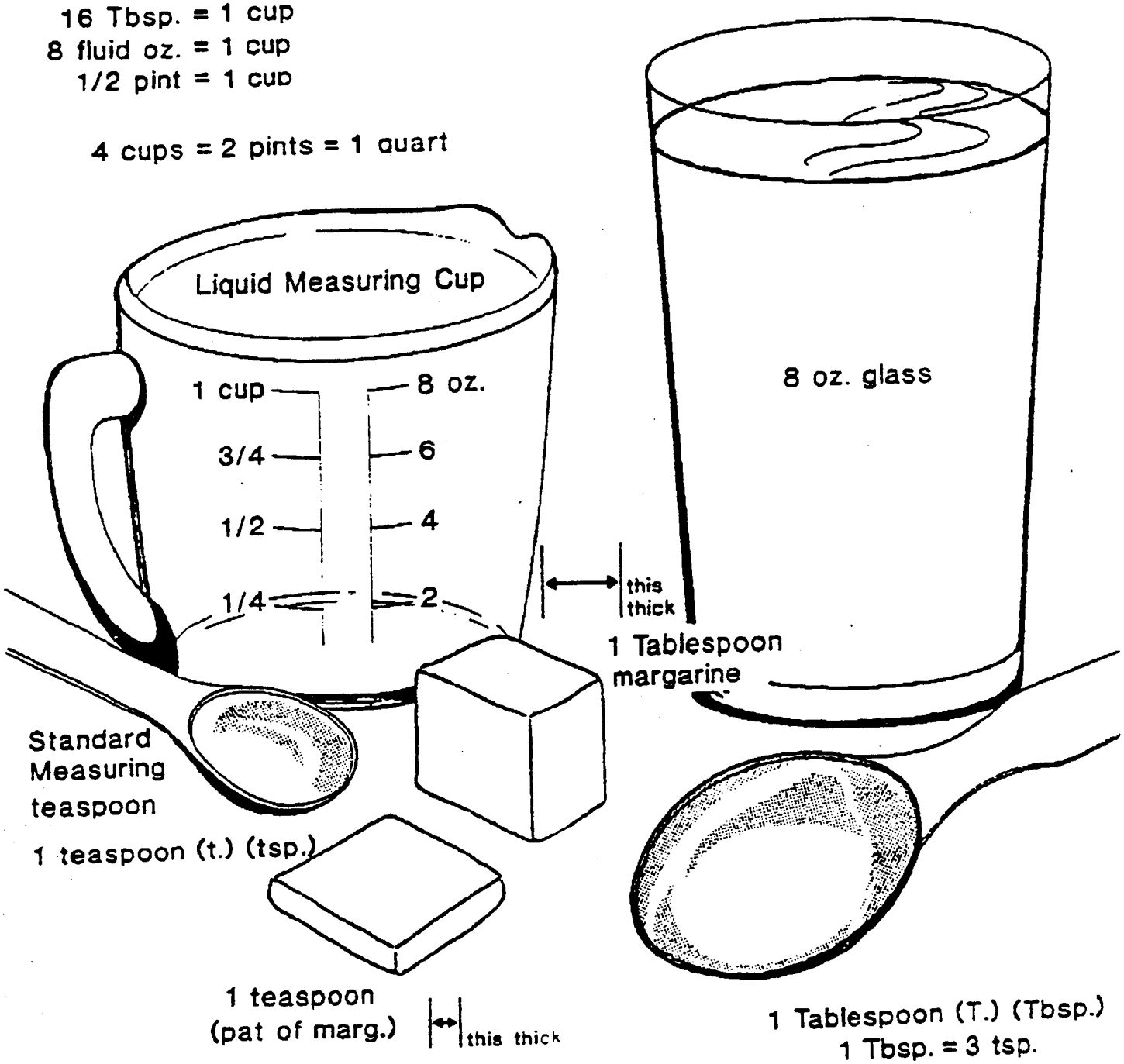


COMMON SERVING SIZES

4 Tbsp. = 1/4 cup
5 1/3 Tbsp. = 1/3 cup

16 Tbsp. = 1 cup
8 fluid oz. = 1 cup
1/2 pint = 1 cup

4 cups = 2 pints = 1 quart



5.7 SPECIAL PROCEDURES FOR DEPARTURES FROM PROTOCOL

Every center will keep a daily diary log book for recording all unusual circumstances involving any of the procedures in diet management, and food production. This log may be used to interpret unusual data points in analysis.

5.7.1 Missed meals

If a participant is absent for a meal, without a valid reason, he/she may be dismissed from the study. If the participant did not eat any non-study food during the absence, a replacement meal may be provided to be eaten during the remainder of the day.

5.7.2 Food that is left

If the participant is still at the feeding site, give any food that is left back to the participant to eat. If the participant cannot eat all of the meal, it may be packaged for later consumption or it may be added to the next meal. Foods that cannot be safely or aesthetically saved for another meal should be discarded and a replacement provided.

5.7.3 Illness

Illnesses that interfere with dietary compliance should be reported immediately to the study director.

5.7.4 Carry out meals

In order to provide flexibility for unusual or very special occasions, each participant will be allowed one additional carry out menu (1 day's food) per diet period. These occasions must be planned in advance.

5.7.5 Missing, lost or spoiled food

If the mishap occurs during the periods of meal service, a replacement item can be provided at the next meal. If the mishap occurs in the evening or on the weekend, the participant is instructed to telephone the person on call. The person on call will either contact the dietitian or provide guidance directly to the participant on appropriate substitutions. Wherever possible, DELTA study food will be provided. Emergency meals may be used here (see section 5.7.6).

5.7.6 Emergency meals

In rare instances, a participant may not be able to get to the feeding site because of an unavoidable, unplanned emergency. Examples of such genuine emergencies might be a critically ill child or household problem requiring the participant to stay at home over a meal period. Field centers may choose to provide an emergency frozen meal pack to each participant as a backup.

However, it is preferable to arrange for delivery of emergency meals to participants in cases where the participant cannot get to the feeding site. Pending natural disasters (hurricanes and snowstorms) usually allow adequate warning for advance preparation. Each field center should have a plan for dealing with these events. (Specific plans for each center follow.)

If an emergency back up frozen entree is provided for each participant, any unused entrees must be returned to the feeding site at the end of each diet period to avoid mixing up of diets. The emergency meal is a Healthy Choice™ meal and accompaniments.

5.7.6.1 Emergency Meal Plans

Columbia

Most of our participants live around the dining facility, so it is convenient for them to come or have their friends pick-up their meals. For those who live far away we pack meals. We also use "free meal" allowance during the week instead of the weekend.

Pennington (PBRC)

At PBRC, advance warning of natural disasters (heavy rain or hurricanes) is expected. In the event of pending flooding, meals will be prepared in advance and packaged for take-out. The participants will take their meals with them before the event occurs. If a participant is unable to leave their home, every effort will be made to deliver the meals to them.

Minnesota

In the event that an overnight major snowfall or severe temperature/wind chill is forecast where individuals are advised to stay in their homes due to danger encountered with travel or exposure, the University of Minnesota DELTA Center will pack meals for the following day. Breakfast and the pre-packed lunch will be sent with participants whose job position would not necessitate their coming to the University the following morning. (A number of employees are considered critical to the function of the University medical departments, and are exempt to such an advisory.) In the case of an overnight snowfall, it would be likely that most participants would be able to come to the center for their evening meal the next day as snow removal takes place in only a matter of hours in this area of the country. Participants who are living long distances from the center AND who would have no other purpose to come to the center will be given their packed breakfast and lunch meal along with Healthy Choice™ entree(s) and the remaining fruit, vegetable, bread and fat items that would have otherwise made up that evening meal. The DELTA kitchen is considered a medical research unit and is exempt to days/shifts canceled due to weather conditions. Employees are available to serve meals for those participants who are willing and able to eat at the center.

Penn State

Two work days prior to each feeding period, the Penn State DELTA-Metabolic-Kitchen staff will prepare and pack one complete Weekend Menu 1 for each participant. On the first day of each feeding period, the packaged meals along with storage instructions will be distributed to the participants. The participants will be allowed to use these extra meals when they are unable to get to the feeding site due to a natural disaster. Any participant who uses the extra meals before the end of the feeding period will be provided with another day's worth of Weekend Menu 1 packaged meals. All unused meals must be returned to the feeding site.

5.7.7 Participant refuses to eat an item

If the participant can no longer tolerate a given menu, then another menu can be substituted for that person. This means the participant would have the same menu twice in a cycle. If the participant can no longer tolerate some of the fruits and vegetables, substitutions can be made for these items only. A list of acceptable substitutions is shown in Figure 1.

NOTE: No substitutions for calcium fortified orange juice are permitted.

Figure 1
LIST OF ACCEPTABLE SUBSTITUTIONS

		Kcal	Pro GM	CHO GM	Fat GM
APPLE JCE, CND/BTL*	100.00 GM	46.94	0.06	11.66	0.11
GRAPE JCE, CND/BTL*	77.000 GM	46.92	0.43	11.50	0.06
PINEAPPLE JCE,CND*	84.000 GM	47.01	0.27	11.55	0.07
CRANBRY JUICE CKTL*	83.000 GM	47.19	0.00	11.92	0.08
ORANGE,ALL,RAW	100.00 GM	46.95	0.94	11.74	0.12
ORANGE,MANDRN,C+JCE	127.00 GM	46.91	0.79	12.13	0.04
GRAPEFRT,PNK/RED,RW	155.00 GM	46.45	0.85	11.89	0.15
GRAPEFRT,CND,LSYR	78.000 GM	46.77	0.44	12.03	0.08
APPLE,W/SKIN,RAW	100.00 GM	58.97	0.19	15.24	0.36
APPLESAUCE,CND,SWT+NA	78.000 GM	58.60	0.14	15.35	0.14
APPLESAUCE,UNSW	137.00 GM	58.87	0.23	15.45	0.07
BANANA,RAW	64.000 GM	58.85	0.66	14.97	0.31
GRAPES,AMER.,RAW	93.000 GM	58.57	0.59	15.92	0.33
FRUIT CKTL,CN,HSYR	80.000 GM	58.34	0.31	15.11	0.06
FRUIT CKTL,CN,LSYR	103.00 GM	58.66	0.41	15.35	0.07
FRUIT CKTL,CN,WTR	183.00 GM	58.49	0.77	15.54	0.09
PEACH,RAW	137.00 GM	58.84	0.96	15.18	0.12
PEACH,CND,HSYR	79.000 GM	58.40	0.36	15.74	0.08
PEACH,CND,JUICE	133.00 GM	58.42	0.84	15.37	0.04
PEAR,RAW	100.00 GM	58.88	0.39	15.08	0.40
PEAR,CND,HSYR	79.000 GM	58.36	0.16	15.13	0.10
PEAR,CND,JUICE	118.00 GM	58.95	0.40	15.26	0.08
PINEAPPLE,RAW	120.00 GM	58.73	0.47	14.82	0.51
PINEAPPLE,CND,HSYR	75.000 GM	58.42	0.26	15.11	0.08
PINEAPPLE,CND,JUICE	98.000 GM	58.72	0.41	15.37	0.08
HONEYDEW,1/10/RAW	168.00 GM	58.70	0.77	15.39	0.17
CANTALOUPE,RAW	84.000 GM	58.72	1.48	14.03	0.47
TOMATO,RED,RAW	100.00 GM	18.97	0.89	4.33	0.21
TOMATO,CND,WHOLE	93.000 GM	18.57	0.86	3.98	0.22
TOMATO STEW,LS	60.000 GM	18.50	0.53	4.23	0.00

(needs to be checked against ETNV)

***May not be substituted for calcium fortified orange juice.**

	Kcal	Pro	CHO GM	Fat GM	GM
CARROTS,RAW	100.00 GM	42.93	1.03	10.10	0.19
CARROTS,CND,DR SOLIDS	185.00 GM	42.50	1.18	10.23	0.35
BEANS,SNAP,RAW	138.00 GM	42.66	2.50	9.83	0.17
BEANS,SNAP,BOILED	122.00 GM	42.68	2.30	9.60	0.34
BROCCOLI,RAW	153.00 GM	42.81	4.55	8.00	0.53
BROCCOLI,FZ,CHPD,CK	153.00 GM	42.76	4.73	8.17	0.18
CAULIFLOWER,RAW	178.00 GM	42.70	3.54	8.74	0.32
CAULIFLOWER,BOILED	178.00 GM	42.64	3.32	8.21	0.30
PEAS,GREEN,BOILED	100.00 GM	83.80	5.35	15.60	0.22
PEAS,GREEN,CND,SLDS	121.00 GM	83.26	5.33	15.10	0.42
PEAS,FROZEN,UNCKD	109.00 GM	83.93	5.67	14.92	0.40
CORN,BOILED	77.000 GM	82.97	2.55	19.30	0.98
CORN,CND,SOLIDS	103.00 GM	83.30	2.69	19.09	1.03
CORN,FROZEN	95.000 GM	83.60	2.87	19.74	0.73
LETTUCE,ICEBERG	100.00 GM	12.98	1.01	2.09	0.19
LETTUCE,BUTTERHEAD	100.00 GM	12.99	1.29	2.31	0.22
LETTUCE,COS/ROMAINE	80.000 GM	12.78	1.29	1.89	0.16
SPINACH,RAW	57.000 GM	12.53	1.63	1.99	0.20
CELERY,RAW	80.000 GM	12.77	0.53	2.90	0.10
PEPPERS,SWT,RAW	51.000 GM	12.73	0.43	2.70	0.23
PEPPERS,SWT,BOILED	70.000 GM	12.58	0.43	2.72	0.23
MUSHROOMS,RAW	51.000 GM	12.74	1.06	2.37	0.21
MUSHROOMS,CND,SOLIDS	54.000 GM	12.95	1.01	2.67	0.16
MUSHROOMS,BOILED	48.000 GM	12.94	1.04	2.46	0.23
ONIONS,RAW	38.000 GM	12.91	0.45	2.78	0.10
ONIONS,BOILED	46.000 GM	12.87	0.41	2.88	0.07
ONIONS,DEHYDRATED	4.0000 GM	12.91	0.36	3.33	0.02

Abbreviations used in the database:

JCE = juice	LSYR = light syrup
CND, Cn = canned	SWT = sweetened
UN = unsweetened	HSYR = heavy syrup
BTL = bottled	WTR = water
ALL = edible portion	LS = low sodium (irrelevant for macronutrient composition)
C+jce = canned in jce	FZ = frozen
PNK = Pink	DR = drained
RW = raw	

Potato/Rice Substitution List for DELTA Weekend Menus

MENU 1	Calorie level	Frozen Potatoes, g	Cooked Rice to Substitute, g
26%	2000 kcal	200	121
26%	1500 kcal	100	60
26%	2500 kcal	227	137
26%	3000 kcal	273	165
26%	3500 kcal	320	194

MENU 1	Calorie level	Frozen Potatoes, g	Cooked Rice to Substitute, g
30%	2000 kcal	190	115
30%	1500 kcal	100	60
30%	2500 kcal	225	136
30%	3000 kcal	260	157
30%	3500 kcal	300	181

MENU 1	Calorie level	Frozen Potatoes, g	Cooked Rice to Substitute, g
37%	2000 kcal	91	55
37%	1500 kcal	65	39
37%	2500 kcal	175	106
37%	3000 kcal	180	109
37%	3500 kcal	210	127

MENU 3	Calorie level	Frozen Potatoes, g	Cooked Rice to Substitute, g
26%	2000 kcal	200	121
26%	1500 kcal	100	60
26%	2500 kcal	250	151
26%	3000 kcal	280	169
26%	3500 kcal	325	197

MENU 3	Calorie level	Frozen Potatoes, g	Cooked Rice to Substitute, g
30%	2000 kcal	182	110
30%	1500 kcal	90	54
30%	2500 kcal	229	138
30%	3000 kcal	240	145
30%	3500 kcal	270	163

MENU 3	Calorie level	Frozen Potatoes, g	Cooked Rice to Substitute, g
37%	2000 kcal	110	67
37%	1500 kcal	80	48
37%	2500 kcal	190	115
37%	3000 kcal	200	121
37%	3500 kcal	230	139

5.8 QUALITY CONTROL

DELTA will implement a standard procedure for assessing compliance and for monitoring the composition of the experimental diets during the study.

5.8.1 Training

Each center is responsible for implementing a training program for food production personnel. This training will encompass all procedures outlined in Chapter 5 of the DELTA Manual of Procedures (MOP).

5.8.2 Compliance Forms and Instructions

The DELTA Daily Food Record will be filled out and maintained in the field center for possible future data entry. The Packed Meal Form will not be keypunched. Self-reported information from the PMF and snack form is transferred to the DDFR for data entry.

Quality control of participant compliance assessment will require each field center to send copies of a 3.5% random sample of DDFRs and corresponding PMFs to the coordinating center every two weeks. The random sample should be selected and copied by staff who are not involved in the administration of the dietary component and not blinded to the treatment assignment.

5.8.3 Instructions for filling in DELTA DAILY FOOD RECORD (DDFR)

The DDFR is the complete record of each participant's food intake during the entire study. Therefore, each participant should have 162 DDFRs at the end of the study. These forms will be kept in the field centers until such time as it is decided to enter them in the DMS. All data that will be entered are contained in the shaded box on the front page of the DDFR. The DDFRs are color coded for diet (Pink = Diet A, Blue = Diet B, Yellow = Diet C).

NAME: Write in the participant's first and last name.

1. Apply the participant **DELTA ID** label.

2. Write the date of the day the menu will be served.

3. Write the day of the week the menu will be served.

4. This field is pre-printed on the form. Check the menu number against the master menu schedule and check the diet designation and calorie level in the participant's record to be sure the correct form has been selected.

5, 6, 7, 11.

These fields are filled in after the entire days food intake has been accounted for.

BREAKFAST

As each item is assembled on the participant's tray, check the appropriate box in the Assembled/Served column. If **BREAKFAST** was a packed meal, also fill out the corresponding meal on the **PACKED MEAL** (PMF) Form.

When the tray is returned to the service area, examine the containers and check the appropriate box only for foods not completely eaten. If **BREAKFAST** was a packed meal, this column will not be filled in until the participant returns for the next meal. Information will be transferred from the Packed Meal Form. If any part of the meal was packaged up to be eaten later, write in each item under the **LUNCH** menu and do not check the "not eaten" boxes for **BREAKFAST**. If a replacement was made, it should be written in the meal section where it was added.

The comment section may be used to note what was done with the "left" food.

8. Check whether this meal was packed or eaten on site.

9. Enter the code of the food server and the person who checked the meal back.

LUNCH, DINNER

Follow the same procedure as **BREAKFAST**. If **LUNCH** was a packed meal, check each item as it is assembled. Then fill out the **PACKED MEAL** Form for the corresponding meal. Food will not be checked back until the participant returns the packed meal form. Answer questions 8 and 9.

SNACK

The snack will always be a packed meal. Follow procedure for other packed meals. Check back items using the participant's self report from the **SNACK FORM** list when the participant returns the following day.

UNIT FOODS

Check the appropriate box and enter the number of units for each unit food provided. Check back using participant's self report from the **PACKED MEAL** form. Record the total number of Unit Foods consumed in item #11 on the front of the DDFR. Participants are expected, but not required, to consume all of the unit foods provided once they are stabilized on a calorie level.

NON STUDY-PROVIDED FOODS

This section is filled in from self reported information on the **PACKED MEAL and SNACK** forms which will be obtained on the following day when the participant returns for breakfast. Items eaten at the self-selected meal (if used by participant) should be listed on the Packed Meal Form by the participant and then transferred to the back of Saturday's DDFR by study personnel.

CODING

When all study-provided foods have been checked back, count the number of boxes checked as not completely eaten and enter this number in the box for item 5. If no boxes were checked, enter "0". Count the number of boxes checked for breakfast, lunch, dinner and snack only. **Do not include the number of unit foods in this total**.

For coding non-study provided foods, evaluate each food item recorded and enter "0" in the code box if the item was non-caloric, and "1" for caloric items. Sum the numbers in the **Total** box and enter this number in item 6.

Alcoholic beverages should be listed as number of drinks. Enter the number for each kind of alcoholic beverage reported and sum the numbers in the **Total** box. Enter this number in item 7.

If no additional foods or alcoholic beverages were reported, enter "0" for items 6 and 7.

DAILY FOOD RECORD (DDFR)

Name _____

1. DELTA I.D. _____ 2. Date ___ ___ ___ (mm dd yy) 3. Day of Week _____ 4. Menu 1, 2000 Kcal Diet A 5. No. of study items not eaten ☐☐

6. Other items ☐☐ 7. No of alcoholic beverages ☐☐ 8. Was BREAKFAST packed? Yes ☐ No ☐ 9. Was LUNCH packed? Yes ☐ No ☐

10. Was DINNER packed? Yes ☐ No ☐ 11. No. of Unit Foods eaten ☐

BREAKFAST

Assembled/ Served		Check any item not completely eaten*
☐ 124.5g	Orange juice	☐
☐ 28.4g	Bran flakes	☐
☐ 50.4g	PF, Whole Wheat toast	☐
☐ 9.0g	Butter	☐
☐ 2 tsp	Grape jelly (1 pc)	☐
☐ 245.0g	Whole milk	☐
☐	Free beverage	

COMMENTS: _____

_____

_____

Food server code _____ Checker code _____

LUNCH

Assembled/ Served		Check any item not completely eaten*
Turkey sandwich		☐
☐ 50.0g	Sliced turkey breast	
☐ 45.4g	PF sandwich white bread	
☐ 4.5g	Mayonnaise	
☐ ¼ cup	Lettuce	
☐ 21.8g	Ginger cookie	☐
☐ 100.0g	Peach slices	☐
☐	Free beverage	

COMMENTS: _____

_____

_____

Food server code _____ Checker code _____

**If item was added to another meal, do not ✓ the column, write the item in the meal where it was added.*

MENU 1 DIET A 2000 KCAL

DINNER

Assembled/ Served			Check any item not completely eaten*
☐ 1 sv	Sirloin tips/gravy/egg noodles		☐
☐ 100.0g	Green peas		☐
☐ 1 sv	Tossed Salad		☐
Salad Dressing:			☐
☐ 1.5g	Olive oil		
☐ 1 TB	Fat free French dressing		
☐ 37.8g	Country classic roll		☐
☐ 10.0g	Butter on roll		
☐ 108.8g	Applesauce		☐
☐	Free beverage		

COMMENTS: _____

_____

Food server code _____ Checker code _____

SNACK

Assembled/ Served			Check any item not completely eaten*
☐ 54.0g	Trail mix		☐

UNIT FOODS

# of Units			
☐ ____	Banana muffin		☐
☐ ____	Carrot muffin		☐
☐ ____	Roll		☐

COMMENTS: _____

_____

Food server code _____ Checker code _____

If item was added to another meal, do not ✓ the column, write the item in the meal where it was added.

INFORMATION FOR THIS SECTION IS TRANSCRIBED FROM THE *PACKED MEAL* FORM ON THE FOLLOWING DAY.

Were any foods or beverages eaten that were not provided by the DELTA Center (Except for Free Beverages)?

Yes ☐ No ☐. If Yes, please list. List alcoholic beverages separately.

Food Item	Amount	Description	Code	Alcoholic Beverages Type	# of Drinks
_____	_____	_____	☐	_____	_____
_____	_____	_____	☐	_____	_____
_____	_____	_____	☐	_____	_____
		TOTAL	☐☐ Enter this # for Q6	TOTAL	☐☐ Enter this # for Q7

CODING: Count the NO. of items checked as not eaten and enter this NO. in Q5. For non-study foods, code each item as 1.

Revised: January 3, 1994

5.8.4 Instructions for filling in the DELTA PACKED MEAL Form (PMF) and SNACK Form

The **DELTA PACKED MEAL** (PMF) form (page 5-42) or the **SNACK** form (page 5-43) will be used for all carry out foods. These forms will be used by the participant to verify meals and other foods and beverages consumed off site. They will be used by center staff to transcribe relevant information to the **DELTA DAILY FOOD RECORD** form for coding. The **DELTA PACKED MEAL and SNACK** forms will not be keypunched, and will remain at the local site.

Before filling in the form, first verify that the menu number matches the corresponding menu number on the **DDFR**.

Enter the first and last name of the participant, ID number, day and the date on which the food will be eaten. When more than one day's meals will be eaten off site, use a separate form for each day.

Kitchen personnel will check off each item as it is put into the container or bag, and enter the packers code number. Before attaching the PMF to the container, be sure to cut off the corner identifying the diet. A Fresh-Check™ Time-Temperature Indicator (TTI) label will be placed in each cooler or bag that the participant takes off site. Each Participant will be given a copy of the TTI instruction sheet (page 44) and instructed on food safety.

The **PMF** or Snack Form will be attached to the container. Instruct the participant to check off each food item after he/she has eaten it. If an item was not eaten, the participant should note the reason under "comments".

Instruct the participant to list all foods and beverages consumed that were not provided by the center, including alcoholic beverages. The completed **DELTA PACKED MEAL Form and SNACK** Forms are returned to the food service unit at the next on site meal.

PACKED MEAL FORM

MENU 1

Name _____ I.D. _ _ _ _ _ _ _ _ Telephone Contact (___) ____ - _____

Day M T W Th F (Circle day) Date ___ ___ ___ mm dd yy Packed by _____

BREAKFAST		LUNCH		DINNER	
Packed	Eaten Y , N*	Packed	Eaten Y , N*	Packed	Eaten Y , N*
☐ Orange juice	☐ , ☐	☐ Turkey sandwich	☐ , ☐	☐ Sirloin tips in	☐ , ☐
☐ Bran flakes	☐ , ☐	☐ Ginger cookie	☐ , ☐	☐ Gravy with	☐ , ☐
☐ Fat spread	☐ , ☐	☐ Peach slices	☐ , ☐	☐ Noodles	☐ , ☐
☐ Whole wheat toast	☐ , ☐	☐ Free beverage		☐ Green (Eng.) Peas	☐ , ☐
☐ Grape jelly	☐ , ☐			☐ Tossed salad	☐ , ☐
☐ Milk .	☐ , ☐			☐ Salad dressing	☐ , ☐
☐ Free beverage				☐ Country classic roll	☐ , ☐
				☐ Fat spread on roll	
				☐ Applesauce	☐ , ☐
				☐ Free beverage	
*Comment: _____		*Comment: _____		*Comment: _____	

UNIT FOODS			Participant, please list any other foods or beverages you ate other than "free beverages."		
# of Units	Packed	Eaten Y , N*	Item	Amount	Description
_____	☐ Roll	☐ , ☐			
_____	☐ Carrot muffin	☐ , ☐			
_____	☐ Banana muffin	☐ , ☐			
*Comment: _____					

Revised: January 26, 1995 - 925

Snack Form

Menu 2

Name: _____ ID: _____

Day: _____ Date: ___ / ___ / ___
mm / dd / yy

Packed by: _____ Contact Tel. #: ____-____-_____

Packed		Eaten Y , N*
☐	Pudding	☐ , ☐
☐	Vanilla Wafers	☐ , ☐
☐	_____	☐ , ☐
☐	Free beverage	☐ , ☐

*Comment: _____

Participant, please fill any other foods or beverages you ate other than "free beverages."

Item	Amount	Description

Snack Form

Menu 2

Name: _____ ID: _____

Day: _____ Date: ___ / ___ / ___
mm / dd / yy

Packed by: _____ Contact Tel. #: ____-____-_____

Packed		Eaten Y , N*
☐	Pudding	☐ , ☐
☐	Vanilla Wafers	☐ , ☐
☐	_____	☐ , ☐
☐	Free beverage	☐ , ☐

*Comment: _____

Participant, please fill any other foods or beverages you ate other than "free beverages."

Item	Amount	Description

ATTENTION

Dear Participant,

Fresh-Check® Time-Temperature Indicator (TTI) labels are being used to insure the freshness and quality of the food you will be using in the Delta Study. **Please follow the guidelines below for handling foods with *Fresh-Check* labels.**

GUIDELINES FOR HANDLING PRODUCTS WITH FRESH-CHECK LABELS

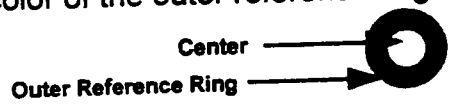
Fresh-Check *labels will change color over a period of 5 days at 40 °F, 1 day at 50 °F and 45 minutes at room temperature. To ensure maximum product shelf life:*

1) BRING FOOD HOME IMMEDIATELY, *do not let food sit in car or out on counter top.*

2) REFRIGERATE FOOD IN COLDEST SECTION OF REFRIGERATOR *(40 °F or cooler).*

HOW TO READ THE FRESH-CHECK LABEL

The ***Fresh-Check*** Indicator is read by comparing the color of the indicator center to the color of the outer reference ring.



1. If the indicator "center" is lighter than the outer reference ring, ***THE FOOD IS FRESH.*** *See example below.*

2. If the indicator matches the outer ring, ***FOOD IS STILL FRESH BUT APPROACHING EXPIRATION AND SHOULD BE USED IMMEDIATELY.*** *See example below.*

3. If the indicator center is darker than outer ring, ***FOOD IS STARTING TO SPOIL AND SHOULD BE DISCARDED.*** *See example below.*

5.8.5 Food Safety

Food safety is a serious concern in every feeding study. In **DELTA**, participants will receive virtually all of their food from the field centers.

Each center is responsible for implementing appropriate procedures and training of personnel to protect participants from any food born illness. "Critical control points are those areas in the chain of food production, from raw materials to finished products, where the loss of control can result in an unacceptable food safety risk."[1]

Critical areas to be addressed include treatment of foods, personal hygiene and health of the food handlers, and misuse of food taken off site by the participants.

Procurement of fresh and wholesome foods that meet study specifications is the first step in food safety. Perishable foods should be dated and refrigerated or frozen immediately upon receipt. Refrigerators must be kept at 34° - 40° F and freezers at 0° or lower. All food should be labelled with date of receipt and stored so that "first in is first out." All foods, cooked or raw, will be stored in closed containers.

Raw meats, poultry and fish require extra safety measures. These food products should be wrapped securely and placed on a tray so they do not leak and contaminate other foods and surfaces. Keep all meats, poultry and fish refrigerated until ready to be cooked. All poultry needs to have the skin removed and be rinsed and patted dry before cooking. All frozen meats, poultry and fish should be thawed in the refrigerator.

Cooked and raw foods must be stored separately. Precision weighed perishable food must be refrigerated immediately after weighing. Cooked foods that are served cold are weighed into individual servings and refrigerated or frozen immediately. Foods that are served hot must be served immediately after cooking. The cooked food should be checked at each meal service to insure that it has reached the proper internal temperature to kill microbial contaminants. (See Table 1). "The time elapsed between tray assembly and delivery is in compliance with food service standards. Tray assembly and delivery time should not exceed 20 minutes to maintain appropriate food temperature, appearance and palatability."[2]

Proper storage of shelf stable food is also crucial in food safety. Foods should be stored off the floor in closed containers, and in well ventilated areas. These storage areas should only be accessible to kitchen staff and **DELTA** personnel. All foods should be labelled with date of receipt and stored so that "first in is first out."

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[1]Adapted from a publication in process by Elaine Ayers, MS, RD, LD, *Metobolic Diet Studies in Humans: A Practical Guide to Design and Management*, P.15

[2]Ayers, op. cit. p.19

Personal hygiene is also critical for food safety. Food handlers should wash their hands with hot soapy water prior to any handling of food items. This same procedure must be followed before **AND** after handling any raw meats and poultry. Any food handler who is sick with a diarrheal illness will not prepare or serve food.

Participants who take food off site also need to follow guidelines to ensure food safety. Meals that will be eaten off site should be packed in insulated bags and coolers. Perishable foods will be kept cold or frozen before they are packed. Keep packed food in cool place and out of direct sunlight until it can be refrigerated or frozen or is ready for consumption.

Each center is responsible for providing information on keeping food safe and insuring that participants have adequate facilities for storing food safely off site. Each participant will be given a copy of "Safe Food to Go".

Table 1: Principles of Time/Temperature Control of Potentially Hazardous Foods[3]

1. Cook food to a minimum temperature:

165°	Poultry and Stuffing
150°	Pork
140°	Other entrees and casseroles

2. Reheating foods for research diets:

165°	minimum

3. Cool foods (liquid formulas) rapidly to 45° in FOUR HOURS using:

Shallow pans (2-3" depth)
Ice bath
Agitation
Loose fitting covers
No stacking
Placement of food in coldest part of cooling unit

4. Equipment maintenance

Refrigeration units 35° F - 45° F
Freezer units 0° F or below

5. Provide thermometers to be used for checking foods for proper and safe temperatures.

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[3]Ayers, op. cit. p.15

5.8.5.1 Safe Food to Go

Millions of meals and snacks are carried to work and school every day. Whether you call it brown-bagging, brief-casing, or dashboard dining, it's the way lots of us eat when we're away from home. It's a handy and often cost-effective way to eat, but is extremely important to keep those meals safe from bacteria that cause foodborne illnesses.

Food poisoning is caused by bacteria that are widely present in land and aquatic environments, on humans, animals, and birds. If they are in or on food, bacteria can multiply quickly at warm temperatures (between 60 and 125° F). The bacteria that cause most cases of foodborne illness include salmonella, Campylobacter jejuni, Staphylococcus aureus, Clostridium perfringens, and Shigella. Some of them cause illness when sufficient numbers are present in food and multiply in the body after we eat the food; others produce toxins in the food that cause illness after we eat them. There are differences among these bacteria in the way illness develops and the time required for symptoms to occur. The symptoms, however, are similar and include severe abdominal cramps and diarrhea, nausea, and sometimes vomiting. Healthy adults will usually recover in a few days, but these illnesses can be life-threatening for elderly persons, young children, or immunocompromised individuals.

The most common foodborne illnesses are caused by the mishandling of food in our homes or in commercial settings such as food services and other retail food distribution sources. When you are carrying food it is possible to prevent the growth of bacteria by paying attention to temperature control and cleanliness.

Ways to Control Food Temperature

Use insulated bags or coolers. Unless the food is going to be eaten within 2 hours of cooking or refrigeration, you will need a way to keep it very hot or cold. Insulated bags or coolers are available in many sizes. Use ice or a reusable freeze-pack to keep foods cold for several hours.

- Be sure foods are cold or frozen before you place them in the cooler or a cold thermos. If you don't have a cooler, simply freezing a sandwich will help keep it safer. Preparing carried lunches the night before and refrigerating them will help you be sure that the foods are sufficiently chilled before packing.

- Keep the lunch bag or cooler in the coolest possible place. For example, a car trunk might be the coolest place in the winter, but in the summer, it will be very hot. In hot weather, keep a cooler (or andy perishable food) inside the car, preferably on the floor. Keep foods out of direct sun where higher temperatures will develop.

- Put frozen yogurt cups or drink boxes in lunch bags to keep foods cool; by lunchtime, they'll be defrosted but still cool.

Use wide-mouth thermos containers for hot foods. Rinse the thermos with very hot water before you fill it with hot foods. Soups, sloppy joe mix, taco filling, stews, hot dogs in chili or hot water, and even casserole mixtures can be safely carried this way, provided they are extremely hot (above 165°F) when you fill the thermos. A good thermos will keep food at safe temperatures for several hours.

Safe Food to Go
To protect your foods when on the go: ■ Pack foods in a refrigerated, frozen or hot condition. ■ Use insulated bags, coolers, or wide-mouth thermoses to keep foods cold or hot. ■ Check "use by" dates on processed meats and poultry, and on deli selections. ■ Wash fruits and vegetables carefully before packing and use clean utensils when preparing foods.

Shop for Lunch Foods with Safety in Mind

Check "use by" dates when you buy processed meats and poultry. This will help you choose sandwich ingredients that are fresh.

If you buy pre-sliced or shaved meats from bulk deli displays, plan to use them within 1 or 2 days. Storage times for these meats are more limited than for prepackaged lunch meats.

If you have no way to keep meat, poultry, or fish ingredients cold, buy forms that do not need refrigeration. Canned meats, meat spreads, entrees, poultry products, or fish will be safe to eat without refrigeration. Just open and use, or heat before eating. However, discard unused portions if you can't refrigerate them. Fermented and cured sausages like hard salami, pepperoni, beef sticks, and jerky are safe without refrigeration.

Natural cheeses are also safe, but may become oily on the surface. And, of course, there is no potential microbial hazard in a peanut butter sandwich!

Single serving portions may be best to buy if you can't refrigerate leftovers.

If you buy food from a refrigerator or freezer case, remember that you need to keep it cold until you eat it.

Deli salads, entrees, and sandwiches that you purchase for carried lunches should be kept cold constantly until you eat them. Check "use by" dates on foods whenever you can. Buy these foods last and get them home quickly. Take a cooler with you to the store if the weather is hot or if it takes more than an hour to get home.

Careful Preparation Prevents Illness

When you prepare food for lunch, be sure the counter top and all tools you use are clean. One of the ways bacteria get into food is by cross-contamination from unclean cutting boards or utensils.

Wash all raw fruits and vegetables thoroughly before using in food mixtures or packing with lunch. Bacteria from the soil and from repeated handling are on these fresh products, and can easily be removed by thorough washing in cool water.

If you make sandwiches more than a day before they are to be eaten, wrap them securely and freeze them. Most sandwich fillings freeze well. Salad fillings usually made with mayonnaise (which breaks down when thawed) should be made with salad dressing instead. Add lettuce or other vegetables just before eating. If you use salad dressing on a sandwich, remember that the acid in these products actually helps slow down bacterial growth.

When you plan to use leftover cooked meats for lunches, be sure to keep them refrigerated until you use them, and use within 3 or 4 days. For longer storage, freeze leftover meat or poultry.

Homemade soups, casseroles, or entrees carried for microwave heating at work should be packed in clean, covered containers and kept cold until reheated. These foods can be risky unless they are chilled immediately after cooking and used within a day or two. Discard any food that has been at room temperature for more than 2 hours.

When You Can't Do It Right -- Don't Do It

Sometimes it just isn't possible to carry your lunch and keep it cold and safe. If some of the foods that don't require refrigeration aren't appealing to you, it's probably a good idea to eat out, or simply take along a pre-packaged snack food (crackers, cheese, snack bar, trail mix). The cost in lost time, possible medical attention, and physical discomfort is far greater than an occasional purchased lunch. But if you follow the easy suggestions offered, you can pack a lunch and enjoy it without worry, too.[4]

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[4]Adapted from Nutrition Update, Volume III, Issue 11, Summer 1993

DELTA: FOOD SERVICE SELF-INSPECTION

Instructions for filling out form: This form is to be filled out weekly. The Supervisor of each site will inspect the personnel and the facility. Any departures from these guidelines and any items that are checked "NO" must be referred to the Principal Investigator or his/her designate.

Date: _____ Inspected by: _____

Inspection Instructions	Yes	No	Comments
I. *Personal Hygiene and Habits*			
1. Hands clean, fingernails short - washed as frequently as necessary in an approved hand-washing facility.			
2. Employees free of burns, cuts, boils, infection.			
3. Employees free of acute respiratory illness or diarrheal illness or other communicable disease.			
4. Clean outer garments worn at start of shift.			
5. No smoking or eating in food preparation or serving areas.			
6. No rings (except wedding band) or jewelry on hands or wrist.			
7. Hair nets worn. No beards or moustache.			
8. Employees using disposable tissues instead of handkerchiefs.			
9. Hands kept away from mouth, nose, hair, etc.			
II. *Food Preparation, Holding, Handling*			
10. Potentially hazardous food kept below 40° or above 150°F -- not held at room temperature more than 2 hours, cumulatively.			
11. Food preparation area generally clean, free of debris.			
12. Fruits, raw foods and vegetables washed prior to preparation.			
13. Frozen food thawed under refrigeration, in the microwave, under cold running water, or cooked directly -- no refreezing of thawed foods. All frozen meats should be thawed in refrigerator.			
14. Cooked leftovers thoroughly reheated.			
15. Food covered or protected from contamination (dust, sneezing, coughing).			

DELTA: FOOD SERVICE SELF-INSPECTION

Inspection Instructions	Yes	No	Comments
16. Self-service utensils dispensed so that only handles can be touched.			
17. Sugar, condiments, seasonings, dressings for self-service: in individual packets or from protected dispensers.			
18. Raw and cooked or ready-to-serve foods not being prepared on same work surface (cutting board) without washing and sanitizing between changed use.			
19. Food handlers should wear plastic disposable gloves when preparing fresh foods (salads or sandwiches) that will NOT be heated before serving.			
20. Raw meats should be cut on a sanitized cutting board that will not be used for any other ingredients. Hands washed before and after handling raw meat & poultry.			
21. Preparation equipment cleaned and sanitized between changed uses (especially pertains to grinders, slicers, choppers, mixers, knives).			
22. Kitchen equipment clean and properly stored.			
23. Food contact surfaces and utensils free of corrosion, pitting, cracks, crevices.			
24. Thermostats and thermometers accurate and operating.			
25. Stoves, griddles, broilers, fryers, etc., free of grease and properly hooded with filters and vented.			
26. Hood and filters free of accumulation of grease and condensation drippings.			
27. Unused equipment kept clean or removed (equipment not used at least occasionally should not be taking up space in food preparation, service, or storage areas).			
III. *Ware Washing and Storage*			
28. Sufficient hot water to meet washing requirements.			
29. Dishwasher machine water clean; proper amount of detergent used.			
30. Dishes, utensils pre-scraped and rinsed.			
31. Wash temperature kept between 140°F and 160°F.			

DELTA: FOOD SERVICE SELF-INSPECTION

Inspection Instructions	Yes	No	Comments
32. Jets and nozzles in dishwasher kept free of food particles and other obstructions and contaminants.			
33. No overloading or improper racking.			
34. Automatic detergent and rinse dispensers operating.			
35. Rinse temperature at least 180°F being maintained.			
36. Thermometers on dishwasher maintained and operating properly.			
37. Removal from racks and sorting done in sanitary manner.			
38. Wash hands between handling soiled table ware and sanitized ware.			
39. All equipment and utensils air dried -- no toweling. Cutting boards sanitized daily. Keep boards used for meats separate from other cutting boards.			
40. Cleaned and sanitized wares stored off floor in a clean, dry location.			
41. Ware washing equipment cleaned after each day's use to remove chemicals, food particles, soil, debris.			
IV. Storage ***A. Dry (food, equipment, supplies)***			
42. Food stored above floor -- on shelves, racks, or platforms.			
43. Floor clean and free of spilled food and debris.			
44. Shelves high enough -- at least six inches -- to permit cleaning, or area beneath shelves enclosed to preclude dirt.			
45. Shelves clean, durably finished, free of dust and debris.			
46. Food supplies dated upon receipt and stored to insure "first in first out" use.			
47. Stable food (sugar, flour, etc.) stored in containers with tight lids or in original package -- properly identified.			

DELTA: FOOD SERVICE SELF-INSPECTION

Inspection Instructions	Yes	No	Comments
48. Loose and unwrapped food or food where original package is broken stored in pest-proof containers or enclosed in plastic bag and tied -- properly identified.			
49. Single service items stored properly.			
50. Canned goods with large dents segregated for return to distributor.			
51. Area dry -- free from dampness.			
52. Non-food supplies stored separately from food items.			
53. Equipment and supplies stored in neat and orderly manner.			
54. All toxic materials, including any pesticides, properly used from original containers only. When not in use stored in cabinets which are used for no other purpose, or in a place which is outside the food storage, food preparation and cleaned equipment areas.			
55. Cleaning equipment and sanitary chemicals available, properly maintained and stored.			
56. Clean and soiled linens stored separately.			
B. Refrigerator Units			
57. Temperature 40°F or below.			
58. Properly functioning thermometer in each unit.			
59. Clean, free from mold, objectionable odors, spills.			
60. All food stored off the floor in orderly manner.			
61. Storage shelves allow adequate air circulation.			
62. Panned raw or cooked foods, on shelves, covered to prevent contamination.			
63. Open "tin" cans not used for food storage.			
64. Potentially hazardous foods stored in chillable quantities (i.e.; in small, shallow containers).			
65. Leftovers which will not be used within 24-36 hours, frozen.			
66. Raw foods stored apart from and below prepared food.			

DELTA: FOOD SERVICE SELF-INSPECTION

Inspection Instructions	Yes	No	Comments
67. No units overloaded.			
68. Food dated and stored in manner to permit "first in first out" use.			
C. Freezer Units			
69. Properly functioning thermometer in each unit.			
70. Temperature 0°F or below.			
71. Food stored to insure adequate air circulation.			
72. Walls or coils defrosted.			
73. Food off the floor.			
74. Clean, free of debris.			
75. Food labelled with date of receipt and stored in manner to permit "first in, first out" use.			
V. Waste Disposal - Garbage and Trash			
76. Nonabsorbent containers clean (using plastic bags), good repair; paper cartons not being used.			
77. Tight-fitting lids on containers not in continuous (actual) use, or when filled or stored.			
78. Sufficient number of containers to hold waste.			
79. Storage area adequate, clean, and separate from food preparation and food storage areas.			
80. Disposed in approved manner, frequently. Trash containers need to be disinfected weekly.			
VI. General Sanitation			
81. Floors clean, dry (no grease or water); good repair.			
82. Walls, ceilings clean; good repair.			
83. Lighting fixtures clean; good repair, shielding protecting against broken glass; lighting adequate.			
84. Wiping cloths (including sponges) clean, rinsed out in sanitizing solution during use; those used for food contact surfaces restricted to that use.			

DELTA: FOOD SERVICE SELF-INSPECTION

Inspection Instructions	Yes	No	Comments
85. Pests (roaches, flies, rodents, etc.) effectively controlled in all areas.			
86. Equipment with insufficient space provided for easy cleaning behind or between the unit sealed to adjoining equipment or wall.			
87. Electrical and plumbing fixtures maintained and operated properly.			
88. No exposed sewer or water lines over food preparation or storage areas.			
89. Dustless methods used to clean floors and walls at time when least amount of food exposed, except in emergency.			
90. Ventilation systems adequate to control heat, steam, condensation, vapors, fumes; maintained.			
91. Planned sanitary maintenance programs used.			
92. No animals in establishment, except guide dogs.			

5.9 COMPOSITION OF EXPERIMENTAL DIETS

The composition of the experimental diets will be monitored through continuous sampling.

Rationale

The underlying principle for assaying the samples is to verify the composition of the diets actually fed to the participants. Samples sent to FALCC should be an accurate representation of what the participants receive. Food items for the sample should be assembled in a manner identical to that for an actual participant. This means that they are assembled at the same time as the participants' food, and taken from the same source. If food is assembled in a way that identifies it clearly as a monitoring sample, it may not reflect the composition of the participants' diet.

Sampling: Each center prepares 1 menu per day according to the following schedule.

Cycle	COL	LSU	MN	PSU
XX	A-lo	B-lo	C-lo	A
1	A	B	C	A-Hi
2	B-Hi	C-Hi	A-lo	B-lo
3	C-lo	A	B	C
4	A-Hi	B-Hi	C-Hi	A-lo
5	B-lo	C-lo	A	B
6	C	A-Hi	B-Hi	C-Hi
7	A-lo	B-lo	C-lo	A
8	B	C	A-Hi	B-Hi
9	C-Hi	A-lo	B-lo	C-lo
10	A	B	C	A-Hi
11	B-Hi	C-Hi	A-lo	B-lo
12	C-lo	A	B	C
13	A-Hi	B-Hi	C-Hi	A-lo
14	B-lo	C-lo	A	B
15	C	A-Hi	B-Hi	C-Hi
16	A-lo	B-lo	C-lo	A
17	B	C	A-Hi	B-Hi
18	C-Hi	A-lo	B-lo	C-lo
	Key	A = 2,000 Kcal, diet A		
		A-Hi = 3,000 Kcal		
		A-lo = 1,500 Kcal		

In this example, Columbia would prepare Diet A 2000 Kcal 8 menus in cycle 1. In cycle 2 Columbia would prepare Diet B, 3000 Kcal 8 menus and cycle 3, Diet C, 1500 Kcal.

Center specific sampling plans are shown on pages 5-64 through 5-75. The procedure is being developed for sampling the unit foods.

Procedure

1. Identify the correct diet to be sampled (A-hi, A, A-lo; B-hi, B, B-lo; C-hi, C, C-lo) from the sampling schedule.

2. Include this diet as an extra "participant" in your production sheets and production line. All portions for a given food item for this diet/calorie level should be weighed/measured out before being labeled or assembled.

3. The QC sample is assembled at the same time as the participant's meal or snack.

4. Assemble the QC sample by selecting at random each food item for the meal. Be sure you have the correct container. Verify that the correct diet has been sampled.

5. Fill out a DDFR as for a participant and check the boxes in the left column.

6. Add the assembled items to the container.

Problem Resolution

If a center encounters any confusion or difficulty in following this procedure at any time, the Coordinating Center should be notified immediately. The Coordinating Center will provide specific instruction for handling the problem. Samples that do not reflect the participant's diet in amount and kind should be sent to FALCC.

5.9.1 Compositing

At the conclusion of each diet cycle (8 days), the collected daily menus (frozen) for each experimental diet (as described above) will be shipped to the FALCC via overnight delivery. The FALCC will provide all materials and protocols required for food collection and shipping. The FALCC will composite each daily menu into a **diet cycle composite** for each experimental diet from each Field Center. Archive samples of each diet cycle composite will be stored at -60°C for retrospective studies.

The FALCC will do the following assays for protocol 1.

5.9.2 Assays

5.9.2.1 Quality assurance program

The FALCC will determine the total weight, dry weight and total fat of the **diet cycle composite** using rapid assays and a fast turn around time (5 working days from receipt of diet samples).

5.9.2.2 Documentation Program

For documentation purposes The FALCC will assay **diet cycle composites** for total weight, moisture, ash, total fat, cholesterol, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-3 fatty acids, and protein. Total carbohydrate will be calculated by difference. Calories will be calculated using the data from total fat, starch, sugars, and total protein.

The archiving of diet cycle composites of each menu of each diet cycle for each experimental diet at each Field Center provides a key resource to the DELTA participants to: 1. Recheck diet composites for cases where the outcome measurements are unexpected, 2. Validate new and/or alternate analytical methods for the assay of DELTA nutrients, 3. Do retrospective studies on DELTA outcomes for nutrients not on the original assay protocols, 4. Evaluate nutrient data bases for nutrients not studied in the first DELTA feeding study, and 5. Obtain data for designing other dietary intervention studies.

5.9.2.3 Materials

At field center:

- prepared foods from menus
- refrigerator (0-2°C)
- freezer (-20°C or lower)
- heavy paper (e.g. brown paper or newspaper)
- **dry ice (ca. 5 lbs per cooler)**

Food Collection and Shipping Materials (supplied by FALCC):

- Rubbermaid containers (12-cup size) (12 per diet)-**pre-labelled**
- Rubbermaid spatula(s)
- cryogenic marker
- fat-free powder-free gloves (disposable)
- Form #F001 (sample transfer)
- Form #F002 (deviation from SOP)
- thermometer
- shipping cooler(s) (6 menus/cooler)
- packing tape
- Federal Express dry ice identification stickers
- pre-addressed Federal Express shipping labels (1 per cooler)

PROCEDURES

NOTE: The following procedures must be followed exactly. If a deviation occurs, fill out form #F002 and include it with the food shipment.

5.9.2.4 Receipt of Shipping Materials:

Make sure you received all items listed above. If there is a discrepancy or if you should need replenishment of supplies, immediately notify Pam Crvich at (703)231-4361 or FAX (703)231-9070.

5.9.3 Food Collection: Total menu

- READ THROUGH THIS ENTIRE PROCEDURE PRIOR TO FOOD COLLECTION

- PERFORM THE FOLLOWING STEPS FOR EACH MENU OF FOODS TO BE COLLECTED:

- DO NOT MIX LIDS OF CONTAINERS (EACH LID AND CONTAINER IS NUMBERED)

Breakfast

1. Assemble all foods from the breakfast menu. **Include** milk and juices, but **not** *ad lib* beverages (e.g. coffee, tea, water, diet soft drinks).

2. Retrieve a 12-cup Rubbermaid container pre-labelled with the appropriate diet identification and menu identification for the menu you are collecting; enter the date and your initials on the label using the cryogenic marker (supplied).

3. While wearing fat-free powder-free gloves and using a clean Rubbermaid spatula scrape **all** of the food into the container. If bread or a muffin is a part of the meal being collected, set it aside and use it to scrape plate, then add to collection container. Be sure to include any food residues from the spatula by wiping it with the bread or on the side of the collection container.

   NOTE: It is CRITICAL that all food residues are collected, or else analytical values will not reflect the composition of the menu.

4. Completely seal the container, and using the cryogenic permanent marker record the menu ID, date, and your initials on the sample label on the container.

5. Place the container in the refrigerator (0-2°C) until collection of total menu is complete ($\leq$ 24 hrs).

   - READ THROUGH THIS ENTIRE PROCEDURE PRIOR TO FOOD COLLECTION

   - PERFORM THE FOLLOWING STEPS FOR EACH MENU OF FOODS TO BE COLLECTED:

Lunch

1. Assemble all foods from the lunch menu. **Include** milk and juices, but **not** *ad lib* beverages (e.g coffee, tea, water, diet soft drinks).

2. Retrieve the container containing breakfast foods from the same menu from the refrigerator. **CHECK THE LABEL AND MAKE SURE YOU HAVE THE CORRECT CONTAINER FOR THE MENU YOU ARE COLLECTING.**

3. While wearing fat-free powder-free gloves, scrape **all** of the lunch food into the container (use a clean Rubbermaid spatula to obtain **all** food residues; if bread or a muffin is a part of the meal being collected, set it aside and use it to scrape plate, then add to collection container. Be sure to include any food residues from the spatula by wiping it with the bread or on the side of the collection container.)

   NOTE: It is CRITICAL that all food residues are collected, or else analytical values will not reflect the composition of the menu.

4. Completely seal the container, and place it in the refrigerator (0-2°C) until collection of total menu is complete ($\leq$ 24 hrs).

- READ THROUGH THIS ENTIRE PROCEDURE PRIOR TO FOOD COLLECTION

- PERFORM THE FOLLOWING STEPS FOR EACH MENU OF FOODS TO BE COLLECTED:

Dinner

1. Assemble all foods from the dinner menu. **Include** milk and juices, but **not** *ad lib* beverages (e.g coffee, tea, water, diet soft drinks).

2. Retrieve the container containing breakfast and lunch foods from the same menu from the refrigerator. **CHECK THE LABEL AND MAKE SURE YOU HAVE THE CORRECT CONTAINER FOR THE MENU YOU ARE COLLECTING.**

3. While wearing fat-free powder-free gloves, scrape **all** of the dinner food into the container (use a clean Rubbermaid spatula to obtain **all** food residues; if bread or a muffin is a part of the meal being collected, set it aside and use it to scrape plate, then add to collection container. Be sure to include any food residues from the spatula by wiping it with the bread or on the side of the collection container.)

   NOTE: It is CRITICAL that all food residues are collected, or else analytical values will not reflect the composition of the menu.

4. Completely seal the container, and place it in the refrigerator (0-2°C) until collection of total menu is complete ($\leq$ 24 hrs).

- READ THROUGH THIS ENTIRE PROCEDURE PRIOR TO FOOD COLLECTION

- PERFORM THE FOLLOWING STEPS FOR EACH MENU OF FOODS TO BE COLLECTED:

Snacks

1. Assemble all snacks from the menu. **Include** milk and juices, but **not** *ad lib* beverages (e.g coffee, tea, water, diet soft drinks).

2. Retrieve the container for the corresponding breakfast, lunch, and dinner menu items. **CHECK THE LABEL AND MAKE SURE YOU HAVE THE CORRECT CONTAINER FOR THE MENU YOU ARE COLLECTING.**

3. While wearing fat-free powder-free gloves, scrape **all** of the snack food into the container (use a clean Rubbermaid spatula to obtain **all** food residues; if bread or a muffin is a part of the meal being collected, set it aside and use it to scrape plate, then add to collection container. Be sure to include any food residues from the spatula by wiping it with the bread or on the side of the collection container.)

   NOTE: **It is CRITICAL that all food residues are collected**, or else analytical values will not reflect the composition of the menu.

4. Completely seal the container and place it in the **FREEZER** (-20°C or less). All foods from the menu should now be in the container.

5. The food must be **frozen at -20°C or less at least overnight prior to shipment**

5.9.4 Shipping

*** * * * * * DO NOT SHIP ON FRIDAY! * * * * * ***

DO NOT LET PACKED COOLERS SIT AT AMBIENT TEMPERATURE FOR AN EXTENDED TIME PERIOD PRIOR TO FED EX PICK-UP

1. Assemble containers of food to be shipped: **FROZEN solid (at least OVERNIGHT at -20°C) prior to shipment**.

2. Ensure that each container is completely sealed.

3. Fill out a sample transfer form (#F001) for each cooler. Include all required information (see sample form included). Make a copy for your records.

4. **Wrap EACH container of food in several layers of brown paper, newspaper, or other cushioning wrap. This is necessary in order to prevent container breakage during transit.**

5. Place wrapped containers in the cooler, then pack wads of brown paper, newspaper or other cushioning material around each container. **NOTE: A maximum of six (6) containers may be shipped in one cooler. It is most efficient for FALCC if 6 menus per cooler are shipped.**

6. Place a layer of brown paper, newspaper, or other cushioning material on top of containers, then add a minimum of 5 pounds of dry ice. **USE CAUTION WHEN HANDLING DRY ICE; WEAR APPROPRIATE PROTECTIVE APPAREL AND INSULATED GLOVES.**
7. Pack wads of newspaper or brown paper to fill out cooler and prevent movement.

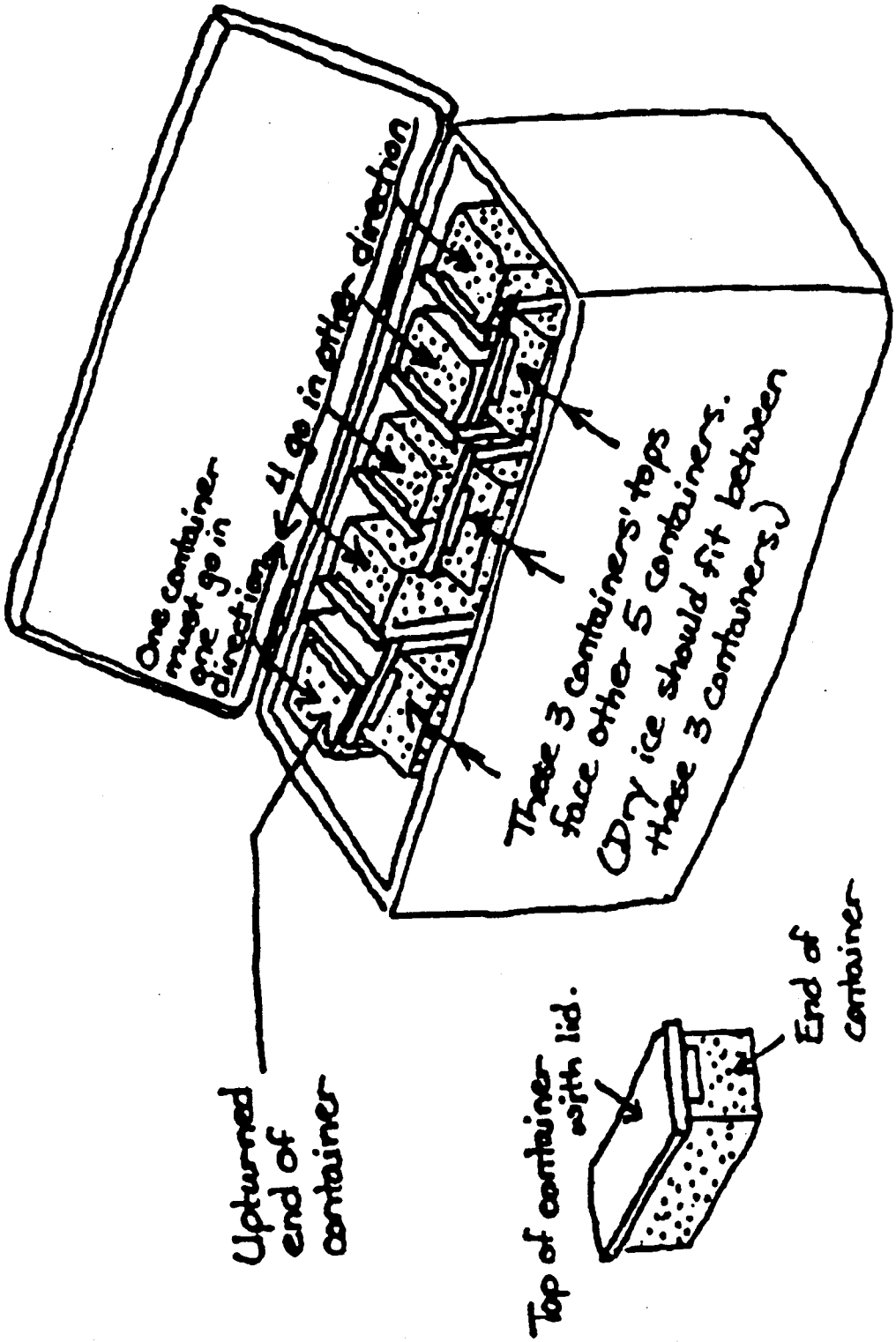
8. Place completed **sample transfer form (#F001)** and Deviation from SOP form(s) (Form #F002, if any) in a sealed zip-lock bag (to protect from moisture), and place in cooler, on top.

9. Tightly seal the lid of the cooler with packing tape as illustrated in Figure 1, (next page). For styrofoam coolers, seal the cooler around the seam as shown, then pack in the cardboard shipping box and seal the box with packing tape.

10. Fill out all information on the dry ice stickers (included in shipping kit) required for Federal Express shipping: Make sure to include your complete address and make sure that the dry ice weight agrees on all stickers for the same cooler.
11. Affix a pre-addressed pre-paid Fed-Ex shipping label to the box, and send via Federal Express overnight delivery to FALCC:
    Dr. K. Stewart
    Dept. of Biochemistry
    304 Engel Hall
    Virginia Tech
    Blacksburg, VA 24061-0308
12. **Notify FALCC of shipment:** Phone: (703) 231-4361 **or** FAX: (703) 231-9070 **or** E-mail: KStewart@VTVM1.CC.VT.EDU

5.9.4.1 Figure 1

Illustration of Shipping Cooler



5.9.4.2
PROTOCOL 1 - FEEDING PERIOD 1

DELTA Sampling Plan for Assays
1993

SEPT.

COLUMBIA

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
			1	2	3	4
5	6	7	8	9	10	11
12	13 Begin Run-in Diet A Menu 5	14 Diet B Menu 6	15 Diet C Menu 1	16 Diet A Menu 2	17 Diet B Menu 3	18
19	20 Diet C Menu 4	21 Diet A Menu 5	22 Diet B Menu 6	23 Diet C Menu 1	24 Diet A Menu 2	25
26	27 XX/3	28 XX/4 A-lo	29 XX/5 A-lo	30 XX/6 A-lo		

Key: Cycle # / Menu #
A-lo: Diet A, 1500 Kcal
Note: Do not sample cycle XX unless directed.

DELTA Sampling Plan for Assays
1993

OCT.

COLUMBIA

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
					1 1 / 1 Begin Sampling A	2 XX / W1 A-lo
3 XX / W2 A-lo	4 1 / 2 A	5 1 / 3 A	6 1 / 4 A	7 1 / 5 A	8 1 / 6 A	9 1 / W3 A
10 1 / W4 A	11 2 / 1 B-hi	12 2 / 2 B-hi	13 2 / 3 B-hi	14 2 / 4 B-hi	15 2 / 5 B-hi	16 2 / W1 B-hi
17 2 / W2 B-hi	18 2 / 6 B-hi	19 3 / 1 C-lo	20 3 / 2 C-lo	21 3 / 3 C-lo	22 3 / 4 C-lo	23 3 / W3 C-lo
3 / W4 24 C-lo 4 / W2 31 A-hi	25 3 / 5 C-lo	26 3 / 6 C-lo	27 4 / 1 A-hi	28 4 / 2 A-hi	29 4 / 3 A-hi	30 4 / W1 A-hi

Key: Cycle # / Menu #
B-lo: Diet B, 1500 Kcal; B: Diet B, 2000 Kcal; C-hi: Diet C, 3000 Kcal; A: Diet A, 2000 Kcal
B-hi: Diet B, 3000 Kcal

DELTA Sampling Plan for Assays
1993

NOV.

COLUMBIA

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
	1 4 / 4 A-hi	2 4 / 5 A-hi	3 4 / 6 A-hi	4 5 / 1 B-lo	5 5 / 2 B-lo	6 5 / W3 B-lo
7 5 / W4 B-lo	8 5 / 3 B-lo	9 5 / 4 B-lo	10 5 / 5 B-lo	11 5 / 6 B-lo	12 6 / 1 C	13 6 / W1 C
14 6 / W2 C	15 6 / 2 C	16 6 / 3 C	17 6 / 4 C	18 6 / 5 C	19 6 / 6 C	20 End of Period 1
21	22	23	24	25	26	27
28	29	30				

Key: Cycle # / Menu #
B-hi: Diet B, 3000 Kcal; C-lo: Diet C, 1500 Kcal; A-hi: Diet A, 3000 Kcal

PROTOCOL 1 - FEEDING PERIOD 1

DELTA Sampling Plan for Assays
1993

SEPT.

PENNINGTON

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
			1	2	3	4
5	6	7	8	9	10	11
12	13 Begin Run-in Diet A Menu 5	14 Diet B Menu 6	15 Diet C Menu 1	16 Diet A Menu 2	17 Diet B Menu 3	18
19	20 Diet C Menu 4	21 Diet A Menu 5	22 Diet B Menu 6	23 Diet C Menu 1	24 Diet A Menu 2	25
26	27 XX/3	28 XX/4 Begin Sampling B-lo	29 XX/5 B-lo	30 XX/6 B-lo		

Key: Cycle # / Menu #
B-lo: Diet B, 1500 Kcal

DELTA Sampling Plan for Assays
1993

OCT.

PENNINGTON

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
					1 1 / 1 B	2 XX / W1 B-lo
3 XX / W2 B-lo	4 1 / 2 B	5 1 / 3 B	6 1 / 4 B	7 1 / 5 B	8 1 / 6 B	9 1 / W3 B
10 1 / W4 B	11 2 / 1 C-hi	12 2 / 2 C-hi	13 2 / 3 C-hi	14 2 / 4 C-hi	15 2 / 5 C-hi	16 2 / W1 C-hi
17 2 / W2 C-hi	18 2 / 6 C-hi	19 3 / 1 A	20 3 / 2 A	21 3 / 3 A	22 3 / 4 A	23 3 / W3 A
3 / W4 A 24 / 4 / W2 B-hi 31	25 3 / 5 A	26 3 / 6 A	27 4 / 1 B-hi	28 4 / 2 B-hi	29 4 / 3 B-hi	30 4 / W1 B-hi

Key: Cycle # / Menu #
B-lo: Diet B, 1500 Kcal; B: Diet B, 2000 Kcal; C-hi: Diet C, 3000 Kcal; A: Diet A, 2000 Kcal
B-hi: Diet B, 3000 Kcal

DELTA Sampling Plan for Assays
1993

NOV.

PENNINGTON

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
	1 4 / 4 B-hi	2 4 / 5 B-hi	3 4 / 6 B-hi	4 5 / 1 C-lo	5 5 / 2 C-lo	6 5 / W3 C-lo
7 5 / W4 C-lo	8 5 / 3 C-lo	9 5 / 4 C-lo	10 5 / 5 C-lo	11 5 / 6 C-lo	12 6 / 1 A-hi	13 6 / W1 A-hi
14 6 / W2 A-hi	15 6 / 2 A-hi	16 6 / 3 A-hi	17 6 / 4 A-hi	18 6 / 5 A-hi	19 6 / 6 A-hi	20 End of Period 1
21	22	23	24	25	26	27
28	29	30				

Key: Cycle # / Menu #
B-hi: Diet B, 3000 Kcal; C-lo: Diet C, 1500 Kcal; A-hi: Diet A, 3000 Kcal

PROTOCOL 1 - FEEDING PERIOD 1

DELTA Sampling Plan for Assays
1993

SEPT.

MINNESOTA

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
			1	2	3	4
5	6	7	8	9	10	11
12	13 Begin Run-in Diet A Menu 5	14 Diet B Menu 6	15 Diet C Menu 1	16 Diet A Menu 2	17 Diet B Menu 3	18
19	20 Diet C Menu 4	21 Diet A Menu 5	22 Diet B Menu 6	23 Diet C Menu 1	24 Diet A Menu 2	25
26	27 XX/3	28 XX/4 Begin Sampling C-lo	29 XX/5 C-lo	30 XX/6 C-lo		

Key: Cycle # / Menu #
C-lo: Diet C, 1500 Kcal

DELTA Sampling Plan for Assays
1993

OCT.

MINNESOTA

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
					1 1 / 1 C	2 XX / W1 C-lo
3 XX / W2 C-lo	4 1 / 2 C	5 1 / 3 C	6 1 / 4 C	7 1 / 5 C	8 1 / 6 C	9 1 / W3 C
10 1 / W4 C	11 2 / 1 A-lo	12 2 / 2 A-lo	13 2 / 3 A-lo	14 2 / 4 A-lo	15 2 / 5 A-lo	16 2 / W1 A-lo
17 2 / W2 A-lo	18 2 / 6 A-lo	19 3 / 1 B	20 3 / 2 B	21 3 / 3 B	22 3 / 4 B	23 3 / W3 B
3 / W4 B 24 / 4 / W2 C-hi 31	25 3 / 5 B	26 3 / 6 B	27 4 / 1 C-hi	28 4 / 2 C-hi	29 4 / 3 C-hi	30 4 / W1 C-hi

Key: Cycle # / Menu #
C-lo: Diet C, 1500 Kcal; C: Diet C, 2000 Kcal; A-lo: Diet A, 1500 Kcal; B: Diet B, 2000 Kcal

DELTA Sampling Plan for Assays
1993

NOV.

MINNESOTA

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
	1 4 / 4 C-hi	2 4 / 5 C-hi	3 4 / 6 C-hi	4 5 / 1 A	5 5 / 2 A	6 5 / W3 A
7 5 / W4 A	8 5 / 3 A	9 5 / 4 A	10 5 / 5 A	11 5 / 6 A	12 6 / 1 B-hi	13 6 / W1 B-hi
14 6 / W2 B-hi	15 6 / 2 B-hi	16 6 / 3 B-hi	17 6 / 4 B-hi	18 6 / 5 B-hi	19 6 / 6 B-hi	20 End of Period 1
21	22	23	24	25	26	27
28	29	30				

Key: Cycle # / Menu #
C-hi: Diet C, 3000 Kcal; A: Diet A, 2000 Kcal; B-hi: Diet B, 3000 Kcal

PROTOCOL 1 - FEEDING PERIOD 1

DELTA Sampling Plan for Assays
1993

SEPT.

PENN STATE

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
			1	2	3	4
5	6	7	8	9	10	11
12	13 Begin Run-in Diet A Menu 5	14 Diet B Menu 6	15 Diet C Menu 1	16 Diet A Menu 2	17 Diet B Menu 3	18
19	20 Diet C Menu 4	21 Diet A Menu 5	22 Diet B Menu 6	23 Diet C Menu 1	24 Diet A Menu 2	25
26	27 XX/3	28 XX/4 Begin Sampling A	29 XX/5 A	30 XX/6 A		

Key: Cycle # / Menu #
A: Diet A, 2000 Kcal

DELTA Sampling Plan for Assays
1993

OCT.

PENN STATE

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
					1 1 / 1 A-hi	2 XX / W1 A
3 XX / W2 A	4 1 / 2 A-hi	5 1 / 3 A-hi	6 1 / 4 A-hi	7 1 / 5 A-hi	8 1 / 6 A-hi	9 1 / W3 A-hi
10 1 / W4 A-hi	11 2 / 1 B-lo	12 2 / 2 B-lo	13 2 / 3 B-lo	14 2 / 4 B-lo	15 2 / 5 B-lo	16 2 / W1 B-lo
17 2 / W2 B-lo	18 2 / 6 B-lo	19 3 / 1 C	20 3 / 2 C	21 3 / 3 C	22 3 / 4 C	23 3 / W3 C
3 / W4 C 24 --- 4 / W2 A-lo 31	25 3 / 5 C	26 3 / 6 C	27 4 / 1 A-lo	28 4 / 2 A-lo	29 4 / 3 A-lo	30 4 / W1 A-lo

Key: Cycle # / Menu # A: Diet A, 2000 Kcal; A-hi: Diet A, 3000 Kcal; B-lo: Diet B, 1500 Kcal;
C: Diet C, 2000 Kcal; A-lo: Diet A, 1500 Kcal

DELTA Sampling Plan for Assays
1993

NOV.

PENN STATE

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
	1 4 / 4 A-lo	2 4 / 5 A-lo	3 4 / 6 A-lo	4 5 / 1 B	5 5 / 2 B	6 5 / W3 B
7 5 / W4 B	8 5 / 3 B	9 5 / 4 B	10 5 / 5 B	11 5 / 6 B	12 6 / 1 C-hi	13 6 / W1 C-hi
14 6 / W2 C-hi	15 6 / 2 C-hi	16 6 / 3 C-hi	17 6 / 4 C-hi	18 6 / 5 C-hi	19 6 / 6 C-hi	20 End of Period 1
21	22	23	24	25	26	27
28	29	30				

Key: Cycle # / Menu #
A-lo: Diet A, 1500 Kcal; B: Diet B, 2000 Kcal; C-hi: Diet C, 3000 Kcal

PROTOCOL 1 - FEEDING PERIOD 2

DELTA Sampling Plan for Assays
1994

COLUMBIA

JANUARY

SUN	MON	TUE	WED	THU	FRI	SAT
						1
2	3	4	5	6	7	8
9	10 3 / xx Begin Period 2	11 4 / xx	12 5 / xx	13 6 / xx	14 7 - 1 Alo	15 W1 / xx
16 W2 / xx	17 7 - 2 Alo	18 7 - 3 Alo	19 7 - 4 Alo	20 7 - 5 Alo	21 7 - 6 Alo	22 7 - W3 Alo
23 7 - W4 Alo	24 8 - 1 B	25 8 - 2 B	26 8 - 3 B	27 8 - 4 B	28 8 - 5 B	29 8 - W1 B
30 8 - W2 B	31 8 - 6 B					

DELTA Sampling Plan for Assays
1994

COLUMBIA

FEBRUARY

SUN	MON	TUE	WED	THU	FRI	SAT
		1 9 - 1 Chi	2 9 - 2 Chi	3 9 - 3 Chi	4 9 - 4 Chi	5 9 - W3 Chi
6 9 - W4 Chi	7 9 - 5 Chi	8 9 - 6 Chi	9 10 - 1 A	10 10 - 2 A	11 10 - 3 A	12 10 - W1 A
13 10 - W2 A	14 10 - 4 A	15 10 - 5 A	16 10 - 6 A	17 11 - 1 Bhi	18 11 - 2 Bhi	19 11 - W3 Bhi
20 11 - W4 Bhi	21 11 - 3 Bhi	22 11 - 4 Bhi	23 11 - 5 Bhi	24 11 - 6 Bhi	25 12 - 1 Clo	26 12 - W1 Clo
27 12 - W2 Clo	28 12 - 2 Clo					

DELTA Sampling Plan for Assays
1994

COLUMBIA

MARCH

SUN	MON	TUE	WED	THU	FRI	SAT
		1 12 - 3 Clo	2 12 - 4 Clo	3 12 - 5 Clo	4 12 - 6 End Period 2 Clo	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30	31		

PROTOCOL 1 - FEEDING PERIOD 2

DELTA Sampling Plan for Annys
1994

P.B.R.C. *JANUARY*

SUN	MON	TUE	WED	THU	FRI	SAT
						1
2	3	4	5	6	7	8
9	10 3 / xx Begin Period 2	11 4 / xx	12 5 / xx	13 6 / xx	14 7 - 1 Blo	15 W1 / xx
16 W2 / xx	17 7 - 2 Blo	18 7 - 3 Blo	19 7 - 4 Blo	20 7 - 5 Blo	21 7 - 6 Blo	22 7 - W3 Blo
23 7 - W4 Blo	24 8 - 1 C	25 8 - 2 C	26 8 - 3 C	27 8 - 4 C	28 8 - 5 C	29 8 - W1 C
30 8 - W2 C	31 8 - 6 C					

DELTA Sampling Plan for Annys
1994

P.B.R.C. *FEBRUARY*

SUN	MON	TUE	WED	THU	FRI	SAT
		1 9 - 1 Alo	2 9 - 2 Alo	3 9 - 3 Alo	4 9 - 4 Alo	5 9 - W3 Alo
6 9 - W4 Alo	7 9 - 5 Alo	8 9 - 6 Alo	9 10 - 1 B	10 10 - 2 B	11 10 - 3 B	12 10 - W1 B
13 10 - W2 B	14 10 - 4 B	15 10 - 5 B	16 10 - 6 B	17 11 - 1 Chl	18 11 - 2 Chl	19 11 - W3 Chl
20 11 - W4 Chl	21 11 - 3 Chl	22 11 - 4 Chl	23 11 - 5 Chl	24 11 - 6 Chl	25 12 - 1 A	26 12 - W1 A
27 12 - W2 A	28 12 - 2 A					

DELTA Sampling Plan for Annys
1994

P.B.R.C. *MARCH*

SUN	MON	TUE	WED	THU	FRI	SAT
		1 12 - 3 A	2 12 - 4 A	3 12 - 5 A	4 12 - 6 End Period 2 A	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30	31		

PROTOCOL 1 - FEEDING PERIOD 2

DELTA Sampling Plan for Assays
1994
MINNESOTA

JANUARY

SUN	MON	TUE	WED	THU	FRI	SAT
						1
2	3	4	5	6	7	8
9	10 3 / xx Begin Period 2	11 4 / xx	12 5 / xx	13 6 / xx	14 7 - 1 Clo	15 W1 / xx
16 W2 / xx	17 7 - 2 Clo	18 7 - 3 Clo	19 7 - 4 Clo	20 7 - 5 Clo	21 7 - 6 Clo	22 7 - W3 Clo
23 7 - W4 Clo	24 8 - 1 Abl	25 8 - 2 Abl	26 8 - 3 Abl	27 8 - 4 Abl	28 8 - 5 Abl	29 8 - W1 Abl
30 8 - W2 Abl	31 8 - 6 Abl					

DELTA Sampling Plan for Assays
1994
MINNESOTA

FEBRUARY

SUN	MON	TUE	WED	THU	FRI	SAT
		1 9 - 1 Blo	2 9 - 2 Blo	3 9 - 3 Blo	4 9 - 4 Blo	5 9 - W3 Blo
6 9 - W4 Blo	7 9 - 5 Blo	8 9 - 6 Blo	9 10 - 1 C	10 10 - 2 C	11 10 - 3 C	12 10 - W1 C
13 10 - W2 C	14 10 - 4 C	15 10 - 5 C	16 10 - 6 C	17 11 - 1 Abs	18 11 - 2 Abs	19 11 - W3 Abs
20 11 - W4 Abs	21 11 - 3 Abs	22 11 - 4 Abs	23 11 - 5 Abs	24 11 - 6 Abs	25 12 - 1 B	26 12 - W1 B
27 12 - W2 B	28 12 - 2 B					

DELTA Sampling Plan for Assays
1994
MINNESOTA

MARCH

SUN	MON	TUE	WED	THU	FRI	SAT
		1 12 - 3 B	2 12 - 4 B	3 12 - 5 B	4 12 - 6 End Period 2 B	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30	31		

PROTOCOL 1 - FEEDING PERIOD 2

DELTA Sampling Plan for Assays
1994

P.S.U.

JANUARY

SUN	MON	TUE	WED	THU	FRI	SAT
						1
2	3	4	5	6	7	8
9	10 3 / xx Begin Period 2	11 4 / xx	12 5 / xx	13 6 / xx	14 7 - 1 A	15 W1 / xx
16 W2 / xx	17 7 - 2 A	18 7 - 3 A	19 7 - 4 A	20 7 - 5 A	21 7 - 6 A	22 7 - W3 A
23 7 - W4 A	24 8 - 1 Bbi	25 8 - 2 Bbi	26 8 - 3 Bbi	27 8 - 4 Bbi	28 8 - 5 Bbi	29 8 - W1 Bbi
30 8 - W2 Bbi	31 8 - 6 Bbi					

DELTA Sampling Plan for Assays
1994

P.S.U.

FEBRUARY

SUN	MON	TUE	WED	THU	FRI	SAT
		1 9 - 1 Cbi	2 9 - 2 Cbi	3 9 - 3 Cbi	4 9 - 4 Cbi	5 9 - W3 Cbi
6 9 - W4 Cbi	7 9 - 5 Cbi	8 9 - 6 Cbi	9 10 - 1 Abl	10 10 - 2 Abl	11 10 - 3 Abl	12 10 - W1 Abl
13 10 - W2 Abl	14 10 - 4 Abl	15 10 - 5 Abl	16 10 - 6 Abl	17 11 - 1 Bbi	18 11 - 2 Bbi	19 11 - W3 Bbi
20 11 - W4 Bbi	21 11 - 3 Bbi	22 11 - 4 Bbi	23 11 - 5 Bbi	24 11 - 6 Bbi	25 12 - 1 C	26 12 - W1 C
27 12 - W2 C	28 12 - 2 C					

DELTA Sampling Plan for Assays
1994

P.S.U.

MARCH

SUN	MON	TUE	WED	THU	FRI	SAT
		1 12 - 3 C	2 12 - 4 C	3 12 - 5 C	4 12 - 6 End Period 2 C	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30	31		

PROTOCOL 1 - FEEDING PERIOD 3

DELTA Sampling Plan for Assays
COLUMBIA ***APRIL, 1994***

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
					1 *GOOD FRIDAY*	2
3	4 xx-3 Begin Per 3	5 xx-4	6 xx-5	7 xx-6	8 13-1 *A-III*	9 xx-W3
10 xx-W4	11 13-2 *A-III*	12 13-3 *A-III*	13 13-4 *A-III*	14 13-5 *A-III*	15 13-6 *A-III*	16 13-W1 *A-III*
17 13-W2 *A-III*	18 14-1 *B-lo*	19 14-2 *B-lo*	20 14-3 *B-lo*	21 14-4 *B-lo*	22 14-5 *B-lo*	23 14-W3 *B-lo*
24 14-W4 *B-lo*	25 14-6 *B-lo*	26 15-1 *C*	27 15-2 *C*	28 15-3 *C*	29 15-4 *C*	30 15-W1 *C*

KEY: A, B, C = 3000 Kcal
A-FB, B-FB, C-FB = 3000 Kcal
A-lo, B-lo, C-lo = 1500 Kcal

DELTA Sampling Plan for Assays
COLUMBIA ***MAY, 1994***

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
1 15-W2 *C*	2 15-5 *C*	3 15-6 *C*	4 16-1 *A-lo*	5 16-2 *A-lo*	6 16-3 *A-lo*	7 16-W3 *A-lo*
8 16-W4 *A-lo*	9 16-4 *A-lo*	10 16-5 *A-lo*	11 16-6 *A-lo*	12 17-1 *B*	13 17-2 *B*	14 17-W1 *B*
15 17-W2 *B*	16 17-3 *B*	17 17-4 *B*	18 17-5 *B*	19 17-6 *B*	20 18-1 *C-III*	21 18-W3 *C-III*
22 18-W4 *C-III*	23 18-2 *C-III*	24 18-3 *C-III*	25 18-4 *C-III*	26 18-5 *C-III*	27 18-6 *C-III* End of Study!	28
29	30 *HOLIDAY!*	31				

KEY: A, B, C = 3000 Kcal
A-FB, B-FB, C-FB = 3000 Kcal
A-lo, B-lo, C-lo = 1500 Kcal

PROTOCOL 1 - FEEDING PERIOD 3

DELTA Sampling Plan for Assays
APRIL, 1994

P.B.R.C.

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
					1 *GOOD FRIDAY*	2
3	4 xx-3 Begin Per 3	5 xx-4	6 xx-5	7 xx-6	8 13-1 *B-HI*	9 xx-W3
10 xx-W4	11 13-2 *B-HI*	12 13-3 *B-HI*	13 13-4 *B-HI*	14 13-5 *B-HI*	15 13-6 *B-HI*	16 13-W1 *B-HI*
17 13-W2 *B-HI*	18 14-1 *C-lo*	19 14-2 *C-lo*	20 14-3 *C-lo*	21 14-4 *C-lo*	22 14-5 *C-lo*	23 14-W3 *C-lo*
24 14-W4 *C-lo*	25 14-6 *C-lo*	26 15-1 *A-HI*	27 15-2 *A-HI*	28 15-3 *A-HI*	29 15-4 *A-HI*	30 15-W1 *A-HI*

KEY: A, B, C = 2000 Kcal
A-HI, B-HI, C-HI = 3000 Kcal
A-lo, B-lo, C-lo = 1500 Kcal

DELTA Sampling Plan for Assays
MAY, 1994

P.B.R.C.

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
1 15-W2 *A-HI*	2 15-5 *A-HI*	3 15-6 *A-HI*	4 16-1 *B-lo*	5 16-2 *B-lo*	6 16-3 *B-lo*	7 16-W3 *B-lo*
8 16-W4 *B-lo*	9 16-4 *B-lo*	10 16-5 *B-lo*	11 16-6 *B-lo*	12 17-1 *C*	13 17-2 *C*	14 17-W1 *C*
15 17-W2 *C*	16 17-3 *C*	17 17-4 *C*	18 17-5 *C*	19 17-6 *C*	20 18-1 *A-lo*	21 18-W3 *A-lo*
22 18-W4 *A-lo*	23 18-2 *A-lo*	24 18-3 *A-lo*	25 18-4 *A-lo*	26 18-5 *A-lo*	27 18-6 *A-lo* End of Study!	28
29	30 *HOLIDAY!*	31				

KEY: A, B, C = 2000 Kcal
A-HI, B-HI, C-HI = 3000 Kcal
A-lo, B-lo, C-lo = 1500 Kcal

PROTOCOL 1 - FEEDING PERIOD 3

DELTA Sampling Plan for Assays

MINNESOTA ***APRIL, 1994***

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
					1 *GOOD FRIDAY*	2
3	4 xx-3 Begin Per 3	5 xx-4	6 xx-5	7 xx-6	8 13-1 *C-III*	9 xx-W3
10 xx-W4	11 13-2 *C-III*	12 13-3 *C-III*	13 13-4 *C-III*	14 13-5 *C-III*	15 13-6 *C-III*	16 13-W1 *C-III*
17 13-W2 *C-III*	18 14-1 *A*	19 14-2 *A*	20 14-3 *A*	21 14-4 *A*	22 14-5 *A*	23 14-W3 *A*
24 14-W4 *A*	25 14-6 *A*	26 15-1 *B-III*	27 15-2 *B-III*	28 15-3 *B-III*	29 15-4 *B-III*	30 15-W1 *B-III*

KEY: A, B, C = 2000 Kcal
A-III, B-III, C-III = 3000 Kcal
A-lo, B-lo, C-lo = 1500 Kcal

DELTA Sampling Plan for Assays

MINNESOTA ***MAY, 1994***

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
1 15-W2 *B-III*	2 15-5 *B-III*	3 15-6 *B-III*	4 16-1 *C-lo*	5 16-2 *C-lo*	6 16-3 *C-lo*	7 16-W3 *C-lo*
8 16-W4 *C-lo*	9 16-4 *C-lo*	10 16-5 *C-lo*	11 16-6 *C-lo*	12 17-1 *A-III*	13 17-2 *A-III*	14 17-W1 *A-III*
15 17-W2 *A-III*	16 17-3 *A-III*	17 17-4 *A-III*	18 17-5 *A-III*	19 17-6 *A-III*	20 18-1 *B-lo*	21 18-W3 *B-lo*
22 18-W4 *B-lo*	23 18-2 *B-lo*	24 18-3 *B-lo*	25 18-4 *B-lo*	26 18-5 *B-lo*	27 18-6 *B-lo* End of Study!	28
29	30 *HOLIDAY!*	31				

KEY: A, B, C = 2000 Kcal
A-III, B-III, C-III = 3000 Kcal
A-lo, B-lo, C-lo = 1500 Kcal

PROTOCOL 1 - FEEDING PERIOD 3

DELTA Sampling Plan for Assays
APRIL, 1994

P.S.U.

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
					1 *GOOD FRIDAY*	2
3	4 xx-3 Begin Per 3	5 xx-4	6 xx-5	7 xx-6	8 13-1 *A-lo*	9 xx-W3
10 xx-W4	11 13-2 *A-lo*	12 13-3 *A-lo*	13 13-4 *A-lo*	14 13-5 *A-lo*	15 13-6 *A-lo*	16 13-W1 *A-lo*
17 13-W2 *A-lo*	18 14-1 *B*	19 14-2 *B*	20 14-3 *B*	21 14-4 *B*	22 14-5 *B*	23 14-W3 *B*
24 14-W4 *B*	25 14-6 *B*	26 15-1 *C-HI*	27 15-2 *C-HI*	28 15-3 *C-HI*	29 15-4 *C-HI*	30 15-W1 *C-HI*

KEY: A, B, C = 2000 Kcal
A-HI, B-HI, C-HI = 3000 Kcal
A-lo, B-lo, C-lo = 1500 Kcal

DELTA Sampling Plan for Assays
MAY, 1994

P.S.U.

SUN	*MON*	*TUE*	*WED*	*THU*	*FRI*	*SAT*
1 15-W2 *C-HI*	2 15-5 *C-HI*	3 15-6 *C-HI*	4 16-1 *A*	5 16-2 *A*	6 16-3 *A*	7 16-W3 *A*
8 16-W4 *A*	9 16-4 *A*	10 16-5 *A*	11 16-6 *A*	12 17-1 *B-HI*	13 17-2 *B-HI*	14 17-W1 *B-HI*
15 17-W2 *B-HI*	16 17-3 *B-HI*	17 17-4 *B-HI*	18 17-5 *B-HI*	19 17-6 *B-HI*	20 18-1 *C-lo*	21 18-W3 *C-lo*
22 18-W4 *C-lo*	23 18-2 *C-lo*	24 18-3 *C-lo*	25 18-4 *C-lo*	26 18-5 *C-lo*	27 18-6 *C-lo* End of Study!	28
29	30 *HOLIDAY!*	31				

KEY: A, B, C = 2000 Kcal
A-HI, B-HI, C-HI = 3000 Kcal
A-lo, B-lo, C-lo = 1500 Kcal

5.10 UNIT FOOD SAMPLING

Scope

This procedure applies to collection of samples of unit foods for monitoring of diet composition as part of DELTA Protocol I (1993), Feeding Periods 2 and 3.

Purpose

To describe the procedure for collecting samples from unit food batches prepared for DELTA Protocol I and shipping the samples to FALCC as part of documenting the composition of experimental diets consumed during feeding trials.

Overview

Three unit foods (carrot muffin, banana muffin, bread) may be prepared for each of three diets (C, B, A). Each center chooses which unit foods to prepare, when they are prepared, and the size of batches. This protocol describes the documentation and sampling of unit food batches for analysis at the FALCC.

Each center will maintain a log of unit food batch preparation. Each center will randomly select two units from each batch of each unit food prepared for each diet throughout the study. These batch samples (2 units) will be individually packaged in zip-lock bags labeled with the center name, unit food name, diet, batch preparation date, and sampling date. All samples will be held at -20°C at the field center **until the end of each feeding period,** then shipped frozen on dry ice to the FALCC. At the FALCC, the samples will be composited and assayed according to the DELTA Protocol.

Materials

At Field Center:

unit food samples from all batches of all diets for entire feeding period
freezer (-20°C or lower)
heavy paper (e.g. brown paper or newspaper)
dry ice

Food Collection and Shipping Materials (supplied by FALCC):

Form #F031 (Unit Food Batch Preparation Log)
zip-lock bags
labels
cryogenic marker
fat-free powder-free gloves (disposable)
Form #F001 (sample transfer), with example form filled out
Forms #F002 (deviation from SOP)
shipping cooler
packing tape[1]
Federal Express dry ice identification stickers
pre-addressed Federal Express shipping labels (1 per cooler)

PROCEDURES

NOTE: Follow these procedures exactly. If a deviation occurs in preparation, packaging, ingredients, sampling, shipping, etc., fill out form #F002 and include it with the food shipment.

TAKE THE SAME CARE SERVING AND PACKING FOODS FOR ASSAY AS YOU DO FOR FOODS FOR PATIENTS.

Receipt of Shipping Materials:

Make sure you received all items listed above. If there is a discrepancy or if you should need additional labels or supplies, immediately notify the FALCC at (703) 231-4361, or FAX (703) 231-8070 or E-mail: FALCC@VTVM1.CC.VT.ECU

[1]**NOTE: The FALCC will supply each field center with a reasonable amount of packing tape for shipping foods to the FALCC - please reserve the tape for this use only.**

SHIPPING * * * * * * **DO NOT SHIP ON FRIDAY** * * * * * *

DO NOT LET PACKED COOLERS SIT AT AMBIENT TEMPERATURE FOR AN EXTENDED TIME PERIOD PRIOR TO FED EX PICK-UP

1. Assemble all samples to be shipped: **FROZEN solid (at least OVERNIGHT AT -20°C) prior to shipment**.

2. Ensure that each bag is completely sealed and properly labeled.

3. Fill out a sample transfer form (#F001). Include all required information (see sample form included); **there should be one entry for each sample enclosed in the cooler**. Make a copy of the form for your records.

4. Place all bags in the cooler, then pack wads of brown paper, newspaper or other cushioning, around them.

   USE CAUTION WHEN HANDLING DRY ICE; WEAR APPROPRIATE PROTECTIVE APPAREL AND INSULATED GLOVES.

5. Place a layer of brown paper, newspaper, or other cushioning material on top of containers, then add a minimum of 5 pounds of dry ice.

6. Pack wads of newspaper on brown paper to fill out cooler and prevent movement.

7. Place completed sample transfer form (**#F001**), Deviation from SOP (Form(s) **#F002,** (if any), and a **copy** of the Unit food Batch Preparation Log (Form **#F031**) in a sealed zip-lock bag (to protect from moisture), and place in cooler, on top.

8. Tightly seal the lid of the cooler with packing tape around seam.

9. Fill out all information on the dry ice stickers (included i shipping kit) required for Federal Express shipping: Make sure to include your complete address and make sure that the dry ice weight agrees on all stickers for the same cooler.

10. Affix a pre-addressed pre-paid FedEx shipping label to the box, and send via Federal Express overnight delivery to FALCC:

    Dr. K. Stewart
    Dept. of Biochemistry
    304 Engel Hall
    Virginia Tech
    Blacksburg, VA 24061-0308

11. **Notify FALCC of shipment:** Phone: (703) 231-4361 **or** FAX: (703) 231-9070 **or** E-MAIL: FALCC@VTVM1.CC.VT.EDU

Documentation of Unit Food Batch Preparation

The purpose of batch documentation is to ensure that all batches prepared are sampled and composited for assay. The "Unit Food Batch Preparation Log" serves this purpose.

1. At the time a batch of unit food is prepared, do the following:

   Record the date of preparation, batch ID number (if any), the unit food name, diet, and batch size (e.g. total number of units or total weight), and your initials.

2. Maintain the preparation log at your center, and **send a copy to the FALCC with the shipment of unit foods.**

Food Collection

1. Wear fat-free powder-free gloves (supplied) while collecting foods.

2. Foe each batch of each type of unit food prepared for each diet throughout the feeding period, **randomly select** two (2) units and place them into a zip-lock bag.

   i.e. **There should be only two units in each zip-lock bag**

3. For each sample (bag), enter the name of your Center, the unit food name, Diet (A, B, or C), batch preparation date, sampling date (=current date), and your initials into the appropriate spaces on a cryogenic label.

4. Affix label to each bag, **INSIDE**.

   NOTE: It is very important to place the label inside the bag, as the label may detach at -20°C.

5. Seal each bag completely and check seal integrity.

6. Freeze and hold all samples at -20°C or lower prior to shipment.

Please return any unused labels and ziplock bags to the FALCC at the end of the feeding period (thank you).

5.11 UNIT FOOD SAMPLING AND ASSAY PLAN

Collection of Unit Food Samples

Each center will maintain a log of unit food batch preparation, which includes the date of preparation, unit food name, diet, and size of batch,. Each center will randomly select one unit from each batch of each unit food (i.e.; banana muffin, carrot muffin, bread) prepared. Each of these units will be individually packaged in an airtight zip-lock bag with the following: center name, unit food name, diet, and batch preparation date. The unit food samples for each feeding period will be held at -20°C at the field centers until the end of that feeding period, when they will be shipped frozen on dry ice to the FALCC along with a copy of the batch preparation log sheet.

Compositing

The FALCC will ensure that a sample has been received from each unit food batch (entered on the log sheet). Unit food composites will be prepared according to the following sampling plan;

	Period I			Period II			Period III		
	Time 1	**Time 2**	**Time 3**	**Time 1**	**Time 2**	**Time 3**	**Time 1**	**Time 2**	**Time 3**
Center 1	U(*,A)	U(*,B)	U(*,C)	U(*,B)	U(*,C)	U(*,A)	U(*,C)	U(*,A)	U(*,B)
Center 2	U(*,B)	U(*,C)	U(*,A)	U(*,C)	U(*,A)	U(*,B)	U(*,A)	U(*,B)	U(*,C)
Center 3	U(*,C)	U(*,A)	U(*,B)	U(*,A)	U(*,B)	U(*,C)	U(*,A)	U(*,B)	U(*,C)
Center 4	U(*,A)	U(*,B)	(U*,C)	U(*,A)	U(*,B)	U(*,C)	U(*,B)	(U*,C)	U(*,A)

There are a total of 12 unit food composites per feeding period. Each composite is prepared from three units of each type of unit food, randomly selected** from the collection of all batch samples of the unit foods prepared at the given center for the indicated diet during the specified time period (above). This means that the size of an individual composite would range from three units (only one type of unit food used) to 9 units (all three unit foods used).

Assay

Each unit food composite will be assayed for proximates (total fat, moisture, protein, ash). Total fat as a percent of kcal will be reported. The fat blends used for unit food preparation will be assayed separately for fatty acid composition.

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*Indicates that the different unit foods would be combined into one composite (by diet, center, and time, above); A, B, and C denote the three different diets; Periods I, II, and III are the three Protocol I feeding periods; Times 1, 2, and 3 are specified timepoints, e.g., weeks 3, 5 and 7 of the period.

**All unit food sample bags for a given diet and time period will be mixed together and the three samples to be composited will be blindly selected.

UNIT FOOD BATCH PREPARATION LOG
CENTER_____

Batch Prep. Date	Internal I.D. No. (if any)	Unit Food Name	Diet (A,B,C)	Batch Size	Initials

CHAPTER 6

FOOD ANALYSIS

TABLE OF CONTENTS

6.0 FOOD ANALYSIS PROCEDURES

DELTA Protocol I

Food Collection and Shipping

The FALCC will supply each field center with the materials and SOP for collection and shipping menus and unit foods. Food collection containers will be pre-labeled and pre-weighed. The food collection and shipping procedure should be followed exactly at each field center. Each field center will be requested to report any deviations to the FALCC, which will document such occurrences. All shipments of food will be made with either dry ice or frozen (-20°C) cold packs via overnight delivery.

Log-in

Each container of food will be weighed, and the weight will be logged into the FALCC sample database. All samples will be stored at -20°C or lower until composited.

Compositing

Each food sample (menus and unit foods) will be composited according to the appropriate Standard Operating Procedure for that sample:

Diet validation. For diet validation, each menu and each unit food for each diet (prepared in duplicate) will be individually composited.

Monitoring. Each diet cylce throughout the feeding study, a diet cycle composites will be prepared for each diet.

Sample distribution

All composited samples will be stored at -60°C or lower:

Archive samples

Archive samples will be stored for the duration of the DELTA study. Place five (5) samples (see Appendix C) of each composite into the archive location designated by supervisor. The dispensation of these samples requires approval by the Steering Committee.

Assay samples

Assay samples will be used for the nutrient assays specified below. Any samples that remain after completion of the nutrient assays will be retained for a minimum of 3 months from the completion of Protocol I.

Reserve samples
Reserve samples are extra samples taken by FALCC to accomodate repeat assays, additional assays, etc. There will be a minimum of eight (8) reserve samples per composite. Reserve samples will be used at the discretion of the FALCC. Any samples that remain after completion of the nutrient assays will be retained for a minimum of 3 months from the completion of Protocol I.

Assay Quality Control

Control samples: For each assay, a control sample (mixed diet composite) will be included with each batch of samples or at least every 20 samples, whichever is less.

QC charts: A QC chart will be established for each assay. Prior to running samples, a minimum of 15 samples of the control material will be assayed, according to the assay SOP. The mean and +/- 2*SD and +/-3*SD will be calculated from these data and plotted as a QC chart. The value for the control material in each assay batch will then be plotted on the QC chart. If the control value falls outside the +/-3*SD limits, the assay will be rejected. The procedure will be evaluated for possible sources of error, corrected if indicated, then samples will be rerun.

Blinded samples: An internal sample numbering system will be used so that the analyst cannot decipher information about a sample from its number. In this way, analyst bias will be minimized. The sample database maintains a link between the sample number and all information concerning that sample. The identity of samples will not be disclosed to the analysts prior to performing assays.

SOPs: FALCC will write Standard Operating Procedures (SOPs) for all nutrient assays and laboratory procedures. Each SOP will be signed and dated. Orginal copies will be kept in a central notebook with disk copies on file as well. Non-current SOPs will be archived.

Assays methods and validation

Total fat

Method: Total lipid will be determined gravimetrically after extraction of the diet composites with chloroform/methanol (modified AOAC 983.23, 1990; see Appendix G4).

Validation: Validation of the method will be based on acceptable recovery of canola oil spiked into composited mixed diets, and acceptable results for standard reference material (NIST SRM #1548[1]). Results will also be compared to data obtained by NCL using acid hydrolysis and modified Folch assays.

[1]National Institute of Standards and Technology (NIST), Gaithersburg, MD

Cholesterol

Method: The method of R. Thompson [Thompson and Merola [USDA Nutrient Composition Laboratory (NCL), Beltsville, MD, 1992], with modifications, will be used to quantify cholesterol (see Appendix G5). The method has been adapted as follows: 1) GC analysis with temperature programming (60°C hold 1 min., then 30°C/min. to 270°C hold for 20 min.) instead of isothermal at 267°C, 2) GC split ratio decreased to 1:20, 3) injection volume decreased to 0.5 μl, 4) cyclohexane added before water in saponification step of sample preparation, 5) 250 μl of of derivatization reagent used.

Validation: Validation of the method will be based on acceptable recovery of cholesterol spiked into mixed diet compoistes, acceptable results for standard reference material (fortified coconut oil, NIST #1563-2), agreement of results with those obtained by Rick Thompson for EZ menus analyzed for Pilot Study #1, and favorable comparison with independent measurements of the cholesterol content of the control sample by NCL.

Moisture

Method: Moisture in the diet composites will be determined with a microwave moisture/solids analyzer (CEM Corp.) (Appendix G6). Moisture will be measured in triplicate for each diet composite. These results will be used to calculate all assay results for the composite on a dry weight basis.

Validation: Validation of the method will be based upon favorable comparison of results by microwave drying (FALCC SOP #5007, App. G6) with those from conventional vacuum oven drying (AOAC 934.01, modified; Appendix G7), acceptable results obtained for NCL reference sample #Q93-FR-4495 used in Pilot Study #1 assays of EZ menus, and favorable comparison of FALCC moisture data for the control sample with those obtained independently by NCL.

Fatty acids (saturated, monounsaturated, polyunsaturated, omega-3)

Method: Fatty acid composition will be determined by gas-liquid chromatography of fatty acid methyl esters prepared from the saponified lipid extracts of diet composites (chloroform/methanol extracts, as prepared for total fat, Appendix G4) (AOCS Official Method Ce 1b-89; 1991, modified). The AOCS method has been adapted to separate and quantitate C:10 and higher fatty acid methyl esters (FAMEs) by lowering the initial temperature (from 170°C to 150°C) (see Appendix G8). The injection volume was also decreased. FAMEs will be reported as triacylglyerol equivalents (TAGs) according to the classification scheme shown in. Fatty acids as TAGs will be normalized to the total fat content (determined as described above).

Validation: Validation will be based upon acceptable fatty acid recoveries from canola oil spiked into diet composites, and favorable comparison of FALCC data for the control sample with those obtained independently by NCL.

Ash

Method: Ashing will be accomplished by vacuum-drying wet diet composite samples, then heating until at 550C in a muffle furnace until completely ashed (Appendix G10).

Validation: Validation will be based upon acceptable ash content determined for NIST SRM #1548, and favorable comparison of FALCC ash data for the control sample with those obtained independently by NCL.

Protein

Method: Protein will be determined as Kjeldahl nitrogen x 6.25. FALCC will subcontract Kjeldahl assay to the Dept. of Human Nutrition and Foods (HNF) at Virginia Tech, which has a semi-automated system. FALCC control samples (blinded) and HNF internal QC samples will be included in each assay batch.

Validation: Validation of the method will be based on acceptable results obtained for NIST SRM #1548, and favorable comparison of results for the control sample with data obtained independently by NCL.

Dietary Fiber

Method: AOAC method 991.43 will be used to measure total dietary fiber. The procedure will be modified for the assay of wet homogenized diet samples instead of ground freeze-dried samples (see Appendix K).

Validation: Validation of the method is based on acceptable results for NIST SRM #1548, and AOAC dietary fiber collaborative study samples[2]. Any modifications to the AOAC method will be validated in this way as well.

[2]Lee et al., JAOAC Intl., **75**: 395-416, 1992.

Data to be reported

component	units
total fat	g/100g dry weight
total SFA	g/100g dry weight (as triglycerides)
total MUFA	g/100g dry weight (as triglycerides)
total PUFA	g/100g dry weight (as triglycerides)
EPA[3]	g/100g dry weight (as triglycerides)
DHA[3]	g/100g dry weight (as triglycerides)
α-L[3]	g/100g dry weight (as triglycerides)
cholesterol	mg/100g dry weight
protein	g/100g dry weight
ash	g/100g dry weight
dietary fiber	g/100g dry weight
moisture	g/100g
total weight	grams
Calculated:	
total dry weight[4]	grams
carbohydrate[5]	g/100g dry weight
total energy[6]	kcal
total fat[7]	% of total kcal
SFA[8]	% of total kcal
MUFA[9]	% of total kcal
PUFA[10]	% of total kcal

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[3]EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; α-L: α-linolenic acid

[4]total wet weight * (1-%H_2O/100)

[5]100 - g protein/100g dry wt - g ash/100g dry wt - g total fat/100g dry wt

[6]total kcal = [g protein/100g dry wt + g CHO/100g dry wt)*4 + (g total fat/100g dry wt)*9] * total dry wt/100

[7] $\frac{\text{(g total fat/100 g dry wt)*9*total dry wt/100}}{\text{total kcal}} *100$

[8] $\frac{\text{(g SFA/100 g dry wt)*9*total dry wt/100}}{\text{total kcal}} *100$

[9] $\frac{\text{(g MUFA/100 g dry wt)*9*total dry wt/100}}{\text{total kcal}} *100$

[10] $\frac{\text{(g PUFA/100 g dry wt)*9*total dry wt/100}}{\text{total kcal}} *100$

Calculated:

component	units
EPA[11]	% of total kcal
DHA[11]	% of total kcal
α-L[11]	% of total kcal

Laboratory Quality Assurance

Instrument/equipment calibration

All balances, instruments, and equipment will be calibrated at least weekly, and more frequently if appropriate. The temperature of refrigerators and freezers is monitored daily. Balances will be calibrated using the protocol and standard weights specified in the DIET MOP.

Audit trail

Because of the large volume of samples and the complexity of processing and assaying samples, the FALCC has developed an internal database to assign numbers and keep track of data concerning any given sample. The database will maintain a link between any given whole food sample and its composited subsamples and all related data and assay results.

Storage of data

Electronic data will be stored on hard drives and floppy disk. Computers will be backed up weekly. Disk copies and hard copies of all data will be archived.

[11] $\frac{(\text{g FA/100 g dry wt})*9*\text{total dry wt}/100}{\text{total kcal}}*100$

6.1 FOOD ANALYSIS LABORATORY CONTROL CENTER STANDARDS

Food Analysis Laboratory Control Center
Standard Operating Procedures

6.1.1 TITLE: Initial Log-in of Food Samples

1. Purpose

To describe the procedure for logging in food samples into FALCC database.

2. Safety

2.1 No specific safety measures are necessary.

3. Materials

3.1 Form F001 (sample transfer, shipped with samples)
3.2 Form F002 (deviation from Standard Operating Procedure) (May or may not be shipped with samples)
3.3 Cryogenic marker (eg. Fisher cat# 13-382-52)
3.4 Sample log-in book
3.5 Red pen
3.6 Computer/printer with Food Analysis Laboratory Control Center (FALCC) Sample Log-in program (M1-33-7)
3.7 Teriwipes(Fisher cat #15-235-60)
3.8 Form F015(Internal Standard deviation form, located in file cabinet #1)
3.9 Freezer (-20°C)

4. Procedure:

4.1 After obtaining permission from QA/QC to proceed with log-in of samples, bring cooler containing samples to Room 304 Engel Hall, Biochemistry from Freezer #3 (Room 102A).

4.2 Make sure the number on the container and lid match; Notify supervisor if numbers do not match each other. Fill out Form F015, internal standard deviation form.

4.3 Turn the computer on. The screen will be in the Windows Program Manager. Select the icon representing Sample Log-in (a swiss army knife); click twice again to select Sample Log-in (Do not select Fox Pro 2.5).

4.4 At the FALCC Main Menu, select Original Sample Log-in.

4.5 At the Sample Input Screen, fill in the following information unless indicated not to: a) **external sample number** (if any, if none, press return)
b) **study name** (window pop-up choices, check with QA/QC for correct choice. use arrow keys to make appropriate choice)
c) **center name** (window pop-up, choices are Penn State, PBRC, Columbia, and Minnesota, check on label for appropriate choice)
d) **menu #** (on label)
e) **cycle #** (See QA/QC for proper choice)
f) **diet** (window pop-up, choices are 1,2,3, other. (Check with QA/QC to determine which repetition or cycle number to enter)
g) **sample description** (window pop-up, check on label for appropriate choice)
h) **date collected** (should be written on label, if none is recorded press enter)
i) **storage location** (window pop-up, check with QA/QC for appropriate choice.)
j) **date received** (record the date cooler arrived at FALCC. (see Form F001))
k) **log-in initials** (your initials).

Look over all information on the log-in screen carefully. If you see a mistake, you can keep hitting the enter key to circle around the screen to correct any mistakes.

4.6 **WRITE THE SAMPLE NUMBER** FROM THE DATABASE ON THE FOOD CONTAINER LABEL AND ON LABEL TAPE USING THE CRYOGENIC MARKER. **WIPE DRY** BEFORE WRITING ON LABEL AND AFFIXING TAPE.

4.7 **ENTER F10 TO SAVE INFORMATION IN DATABASE.**

4.8 Place all containers in cooler, until log-in is complete, to keep cold.

4.9 When you have finished logging-in all containers from any one center, enter F10 to save last information, then ESC to quit. The program will ask if you are ready to print, type in YES. The program will ask you how many copies of print-out do you want. Always print out at least three (3), Check with QA/QC officer to see if more print-outs are needed.

4.10 Make sure each container is tightly sealed, then place all food samples to the freezer (-20°C) storage location designated by supervisor (as entered in database; step 4.5)

4.11 Tape one print-out of log-in information into sample log-in book and return the sample log-in book to the appropriate bookshelf.

4.13 File forms #F001, #F002, #F015 and log-in print outs with QA/QC officer

Prepared by:

Pam Crvich

Date: _____

Approved by:

Katherine Phillips

Date: _____

Food Analysis Laboratory Control Center (FALCC)
Standard Operating Procedure

6.1.2 TITLE: Preparation of Diet Composites

Scope: Procedure applies to total food weight of 1200-2500 grams.

1. Purpose

To describe the procedure for preparing composites of mixed diets.

2. Safety

2.1 Read SOP # 1005 on Use of Robot Coupe model R-6 Batch Processor

3. Materials

3.1 Robot Coupe model R-6 batch processor (M1-7-2, SOP # 1005)
3.2 foods to be composited, frozen (-60°C or less)
3.3 12 X 1" stainless steel spatula (Fisher #14-375-57), or equivalent
3.4 freezers (-20 and -60°C)
3.5 refrigerator (2-8°C)
3.6 fume hood
3.7 thermometer
3.8 straight sided, 30 ml Qorpak glass sample jars with Teflon® lined screw caps (Fisher #03-320-7A), or equivalent
3.9 permanent, **cryogenic** marker (Fisher #13-382-52), or equivalent
3.10 Teri Wipes (Fisher #15-235-60), or equivalent
3.11 timer
3.12 powder-free gloves
3.13 scotch tape
3.14 rectangular pan 10" X 18", filled with ice
3.15 6 quart stainless steel bowl
3.16 10" stainless steel wire whisk
3.17 siliconized Rubbermaid® spatula (SOP # 5008)
3.18 100 ml siliconized tri-cornered polypropylene beakers (Fisher # 02-593-50C), or equivalent
3.19 12" stainless steel scoopula
3.20 small plastic pan for placement under jars while pouring aliquots
3.21 600 ml glass beaker
3.21 analytical balance (M1-39-9, Fisher # 01-913-317), or equivalent
3.22 Synco 2001 Computer (M1-33-5), or equivalent
3.23 Hewlett Packard Laser Jet IIIp printer (M1-39-10), or equivalent

4. Procedure

Note: **Avoid touching anything that may come into contact with the composite. Wear powder free gloves throughout the procedure.**

4.1 Composite the mixed diet

4.1.1 The day prior to compositing, remove the containers of food from the freezer(-20°C). Place the containers containing the diet composites on a bench surface at least 4" away from each other. Let the containers sit out at room temperature for four (4) hours.

4.1.2 Place the containers in the refrigerator (2-8°C) overnight (up to 24 hours) at least 4" away from each other.

4.1.3 On the day of compositing, place the containers containing the diet composites on the bench surface in the fume hood. The containers should be at least 4" apart. Let containers sit out at room temperature for two(2) to four (4) hours.

4.1.4 Prepare station for compositing. Fill the rectangular pan with ice. Twenty-two (22) jars of composite sample will be aliquotted for each diet. Set out sample jars, and number the lids (1-22).

4.1.5 Clean and assemble the Robot Coupe according to SOP # 1005.

4.1.6 **QUANTITATIVELY** and **CAREFULLY** transfer **ALL** of the food from the container into the stainless steel bowl.

4.1.6.1 Weigh the stainless steel bowl. Record the weight on the Composite Worksheet, Form F014.

4.1.6.2 Using a Teri Wipe, wipe off any moisture that has accumulated on the outside of the container. Remove the label from the lid of the plastic container and affix it to the Compositing Worksheet, Form F014. Also affix any other labels adhering to the container to Form F014 (i.e. label tape with noted deviations from total menu). Secure the labels with scotch tape.

4.1.6.3 Using the stainless steel spatula cut the composite into smaller pieces (~3 - 4" across).

4.1.6.4 Using the stainless steel spatula, carefully transfer all of the composite into the stainless steel bowl. **Do not lose any of the composite. If loss occurs, notify supervisor. Complete an Internal Deviation From SOP Form (F015) and file it with the records associated with the diet.**

4.1.6.5 Thoroughly scrape all food residues from the container. Place the side of the spatula against the edge of the container and completely scrape the entire surface. By scraping in this way, you should be able to remove any food residue that is adhering to the sides of the container. Place all residue into the stainless steel bowl. **DO NOT** lose any food on spatula, countertop, etc. Place the spatula, handle side down, in a 600 ml glass beaker.

4.1.6.6 Using the siliconized Rubbermaid® spatula, completely scrape any remaining food residue or droplets from the container into the stainless steel bowl. Place the spatula, handle side down, in a 600 ml glass beaker.

Note: The container should be completely free of any residue. If not notify supervisor.

4.1.7 Weigh the stainless steel bowl and food. Record the weight on Form F014.

4.1.8 Determine the weight of the food by subtracting the weight of the stainless steel bowl (step **4.1.6.1**) from the weight of the stainless steel bowl and food (step **4.1.7**). Record the food weight on Form F014.

4.1.9 **QUANTITATIVELY** and **CAREFULLY** transfer **ALL** of the food from the stainless steel bowl into the bowl of the Robot Coupe. Using the siliconized Rubbermaid® spatula, thoroughly scrape all food residues or droplets from the stainless steel bowl into the bowl of the Robot Coupe. **Do not lose any of the composite. If loss occurs, notify supervisor. Complete deviation from SOP form # F015 and file with the records associated with the diet.** Place the spatula, handle side down, in a 600 ml glass beaker.

4.1.10 Using a Teri Wipe, wipe out the stainless steel bowl (this is to insure that any food residue which does remain, will not be incorporated into the composite when the bowl is used in steps **4.2** and **4.3**).

4.1.11 Scrape down the sides of the Robot Coupe bowl using a stainless steel spatula. Scrape the spatula on the edge of the Robot Coupe blade assembly or gently tap the spatula on the edge of the Robot Coupe bowl. Place the spatula, handle side down, in a 600 ml glass beaker.

4.1.12 Measure the temperature of the food. Record the temperature of the food on Form F014. Place the thermometer, bulb side up, in a 600 ml glass beaker. When the composite reaches 15°C, begin monitoring time. **The composite must not be allowed to remain at 15 - 20°C for more than 30 minutes.**

4.1.13 Set the speed setting on the Robot Coupe to 1000 rpm. Blend the food for 15 seconds by turning on the power switch.

4.1.14 If food has splashed up into the lid of the Robot Coupe, gently tap the lid on the Robot Coupe bowl before removing the lid. After removing the lid, use the siliconized Rubbermaid® spatula to scrape any food material adhering to the lid, back into the Robot Coupe bowl. Scrape the residue off of the siliconized spatula on the side of the Robot Coupe bowl. Place the spatula, handle side down, in a 600 ml glass beaker. Invert the Robot Coupe lid on the bench working surface.

4.1.15 Scrape down the sides of the Robot Coupe bowl using a stainless steel spatula. Scrape the spatula on the edge of the Robot Coupe blade assembly or gently tap the spatula on the edge of the Robot Coupe bowl. Place the spatula, handle side down, in a 600 ml beaker.

4.1.16 Measure the temperature of the composite. Record the temperature on Form F014. Place the thermometer, bulb side up, in a 600 ml glass beaker. When the composite reaches 15°C, begin monitoring time. **The composite must not be allowed to remain at 15 - 20°C for more than 30 minutes.**

4.1.17 Set the speed setting on the Robot Coupe to 1000 rpm. Blend the composite for 15 seconds by turning on the power switch.

4.1.18 Scrape down the sides of the Robot Coupe bowl using a stainless steel spatula. Scrape the spatula on the edge of the Robot Coupe blade assembly or gently tap the spatula on the edge of the Robot Coupe bowl. Place the spatula, handle side down, in a 600 ml glass beaker.

4.1.19 Measure the temperature of the composite. Record the temperature on Form F014. Place the thermometer, bulb side up, in a 600 ml glass beaker. When the composite reaches 15°C, begin monitoring time. **The composite must not be allowed to remain at 15 - 20°C for more than 30 minutes.**

4.1.20 Set the speed setting on the Robot Coupe to 3500 rpm. Blend the composite for 45 seconds by turning on the power switch.

4.1.21 If food has splashed up into the lid of the Robot Coupe, gently tap the lid on the Robot Coupe bowl before removing the lid. After removing the lid, use the siliconized Rubbermaid® spatula to scrape any food material adhering to the lid, back into the Robot Coupe bowl. Scrape the residue off of the siliconized spatula on the side of the Robot Coupe bowl. Place the spatula, handle side down, in a 600 ml glass beaker. Invert the Robot Coupe lid on the bench working surface.

4.1.22 Scrape down the sides of the Robot Coupe bowl using a stainless steel spatula. Scrape the spatula on the edge of the Robot Coupe blade assembly or gently tap the spatula on the edge of the Robot Coupe bowl. Place the spatula, handle side down, in a 600 ml glass beaker.

4.1.23 Measure the temperature of the composite. Record the temperature on Form F014. Place the thermometer, bulb side up, in a 600 ml glass beaker. When the composite reaches 15°C, begin monitoring time. **The composite must not be allowed to remain at 15 - 20°C for more than 30 minutes.**

4.1.24 Set the speed setting on the Robot Coupe to 3500 rpm. Blend the composite for 45 seconds by turning on the power switch.

4.1.25 If food has splashed up into the lid of the Robot Coupe, gently tap the lid on the Robot Coupe bowl before removing the lid. After removing the lid, use the siliconized Rubbermaid® spatula to scrape any food material adhering to the lid, back into the Robot Coupe bowl. Scrape the residue off of the siliconized spatula on the side of the Robot Coupe bowl. Invert the Robot Coupe lid on the bench working surface.

4.1.26 Scrape down the sides of the Robot Coupe bowl using a stainless steel spatula. Scrape the spatula on the edge of the Robot Coupe blade assembly or gently tap the spatula on the edge of the Robot Coupe bowl. Place the spatula, handle side down, in a 600 ml glass beaker.

4.1.27 At this point, the composite should have a uniform, homogeneous appearance and consistency. Using the large, stainless steel scoopula, stir the composite thoroughly and inspect for icy lumps, larger pieces of food, or any inconsistencies with the typical composite appearance. **NOTIFY SUPERVISOR IF ANYTHING LOOKS UNUSUAL.** Tap the scoopula on the edge of the Robot Coupe bowl. Place the scoopula, handle side down, in a 600 ml glass beaker.

4.1.28 Measure the temperature of the composite. Record the temperature on Form F014. Place the thermometer, bulb side up, in a 600 ml glass beaker. **The temperature of the composite must be 15 - 22 °C at this point. Notify supervisor if composite is not in the proper temperature range. If the composite is too cold (i.e. below 15°C), allow the composite to sit in the Robot Coupe bowl at room temperature until the composite temperature is in the desired range. Monitor composite temperature every 15 minutes. Composites which are too warm (i.e. above 22°C), will be handled individually.**

4.1.29 Set the speed setting on the Robot Coupe to 3500 rpm. Blend the composite for 15 seconds by turning on the power switch. **IMMEDIATELY PROCEED TO STEP 4.2.**

Note: No scraping is required in this step.

4.2 Quickly pour the composite from the Robot Coupe bowl into the 6 quart stainless steel bowl that was used in steps **4.1.6 - 4.1.9**.

Note: It is not necessary to completely transfer all of the composite to the stainless steel bowl.

4.3 Aliquot the composite into sample jars

4.3.1 Place four sample jars on the edge of the work space.

4.3.2 Using the stainless steel whisk, stir the composite in the following manner: start stirring at the outer edge of the bowl and work towards the center and then back out again in a smooth, circular motion. Repeat this stirring pattern for a minimum of 15 seconds. Make sure the whisk is touching the bottom of the bowl to insure complete mixing of the composite. Try not to incorporate air while stirring. Proceed immediately to step **4.3.3.**

4.3.3 Using a gloved hand, grasp a siliconized, tri-cornered, polypropylene beaker by one of it's corners at the lip. Quickly dip an aliquot of the composite into the beaker. While holding the jar over the small plastic pan, pour the composite into the prepared sample jars (step **4.3.1**). Fill the jars to approximately two-thirds capacity. There should be enough composite in the beaker to do at least four jars. **Do not hold the jars over the bowl containing the composite while pouring.** Set the beaker aside (this beaker will not be used again). **DO NOT POUR THE COMPOSITE REMAINING IN THE BEAKER BACK INTO THE BOWL CONTAINING THE COMPOSITE.**

4.3.4 Make sure there is no food residue around the threads or on the outside of the jars. Clean the jars with a Teri Wipe if necessary. Cap each sample jar with the appropriately numbered lid, and place the jars in the ice in the previously prepared pan (step **4.1.4**). Keep the samples in the order in which they were aliquotted. The samples must be numbered consecutively.

4.3.5 Measure and the temperature of the composite. Record the temperature of the composite on Form F014. Place the thermometer, bulb side up, in a 600 ml glass beaker. When the composite reaches 15°C, begin monitoring time. **The composite must not be allowed to remain at 15 - 20°C for more than 30 minutes.**

4.3.6 Place four sample jars on the edge of the work space.

4.3.7 Using the stainless steel whisk , stir the composite in the following manner: start stirring at the outer edge of the bowl and work towards

the center and then back out again in a smooth circular motion. Repeat this stirring pattern for a minimum of 15 seconds. Make sure the whisk is touching the bottom of the bowl to insure complete mixing of the composite. Try not to incorporate air while stirring. Proceed immediately to step **4.3.8.**

4.3.8 Using a gloved hand, grasp a **clean**, siliconized, tri-cornered, polypropylene beaker by one of it's corners at the lip. Quickly dip an aliquot of the composite into the beaker. Pour the composite into the prepared sample jars (step **4.3.6**). Fill the jars to approximately two-thirds capacity. There should be enough composite in the beaker to do at least four jars. **Do not hold the jars over the bowl containing the composite while pouring. DO NOT POUR THE COMPOSITE REMAINING IN THE BEAKER BACK INTO THE BOWL CONTAINING THE COMPOSITE.** Set the beaker aside.

4.3.9 Make sure there is no food residue around the threads or on the outside of the jars. Clean the jars with a Teri Wipe if necessary. Cap each sample jar with the appropriately numbered lid and place the jars in the ice in the previously prepared pan (step **4.1.4**). Keep samples in the order in which they were aliquotted. The samples must be numbered consecutively.

4.3.10 Measure the temperature of the composite. Record the temperature of the composite on Form F014. Place the thermometer, bulb side up, in a 600 ml glass beaker. When the composite reaches 15°C, begin monitoring time. **The composite must not be allowed to remain at 15 - 20°C for more than 30 minutes.**

4.3.11 Continue in this way until all 22 samples have been aliquotted.

4.4 Label the sample jars

4.4.1 Turn on the computer by pressing the "on" button on the surge protector box just to the left of the keyboard. The Windows Program Manager screen will appear.

4.4.2 Using the mouse, double click with the left button, on the *Sample Log-in* icon. A sub menu will open. Double click on *Sample Log-in* and the sample log-in main menu will appear.

4.4.3 Using the down arrow key, highlight the *Composite Log-In* block. This block will be the second block in the series. Hit *Enter.*

4.4.4 A screen will appear in which you will enter the date and description of the food to be composited. If not already present, enter the date. The date is in the format MM/DD/YY. Hit *Enter.*

4.4.5 Enter the description of the food to be composited. This description of the food will be on the container holding the food to be composited, i.e. "Total Menu". Hit *Enter*.

4.4.6 A screen will appear in which you will enter the *Source Sample Number* of the food to be composited. The source sample number will be on the container holding the food to be composited. This number will always begin with VT and will be followed by 5 numbers or letters (VTXXXXX). Enter the number. **Carefully** review the entered number and make **sure** it is correct. Hit *Enter*. If this is the only source sample number which will be entered for the food, hit *Esc*. If there is another source sample number to add, enter it in the same way. Hit *Esc* after the last number has been entered.

Note: If an incorrect source sample number is entered, alert Pam Crvich. The database will need to be edited.

4.4.7 A screen will appear which asks the number of samples that will be generated. Enter *22* (this is the number of jars that will be filled for each composite; each jar must have a individual number). Hit *E*nter.

Note: If an incorrect number is entered when asked for the number of samples that will be generated, alert Pam Crvich. The database will need to be edited.

4.4.8 A screen will appear which asks if you are ready to print. Hit *Y* for yes. Hit *Enter*.

4.4.9 A screen will appear which asks how many copies of the Composite Report Form (F009), to print. Enter *4* (one copy will be for the sample log-in notebook, one copy will be given to QA/QC, one copy will be for the compositing notebook, and the fourth copy will be given to Katherine Phillips). Hit *Enter*. Four copies of Form F009 will then be printed. On this form will be the 22 sample numbers which will be used to label the 22 jars used for the composite, and the composite number.

4.4.10 Record the composite number on Form F014.

Note: The jars **must** be labeled with consecutive numbers in the order in which they were aliquotted. Jar #1, the first jar aliquotted, will receive the first sample number generated by the Computer's Sample Log-in process. Jar #2, the second jar aliquotted, will receive the second sample number generated, and so on.

4.4.11 Using a **PERMANENT CRYOGENIC MARKER**, clearly write the composite number (from form F009), the sample number, and date on the top and side of each jar. Zeros should be written ∅ to avoid confusion with the letter "O".

5. Storage

5.1 Make sure jars are tightly sealed. Store at -60°C in the appropriate freezer location in the original boxes in which the samples jars were shipped.

5.2 Record the storage location of the jars in the sample database.

5.2.1 Return to the sample log-in main menu (steps **4.4.1-4.4.2**). Use the down arrow key to highlight *Editing*. Hit *Enter*.

5.2.2 Use the down arrow keys to highlight *Composite Storage Location*. Hit *Enter*.

5.2.3 A screen will appear which asks for the *Source Sample Number*. (This is the same number which was entered in step **4.4.6** for each sample). Enter the number. Hit *Enter*.

5.2.4 A subscreen will appear which asks for the *Composite Storage Location*. This screen will be accompanied by a pop-up screen which has all of the available freezer locations. Use the down arrow keys to highlight the proper location and hit *Enter*.

Note: The screen will then pause, and the entries will disappear. The screen will then be ready for another entry of source sample number and storage location. Continue to enter the information in this way until all entries have been made.

5.2.5 When all entries have been made, hit *Esc* to exit the editing portion of the program and return to the editing menu.

5.3 Record the source sample weight in the sample database.

5.3.1 Use the down arrow keys to highlight *Original Sample Net Weight*. Hit *Enter*.

5.3.2 A screen will appear which asks for the *Source Sample Number*. (This is the same number which was entered in step **4.4.6** for each sample). Enter the number. Hit *Enter*.

5.3.3 A screen will then appear which asks for the *Original Sample Net Weight*. Enter the weight. Hit *Enter*.

Note: The screen will then return to the *Source Sample Number* side for entry of another sample number and weight. Continue to enter the information in this way until all entries have been made.

5.2.4 When all entries have been made, hit *Esc* to exit the editing portion of the program and return to the editing menu.

5.3.5 Use the down arrow keys to highlight *Quit*. Hit *Enter*. The program will then return to the Windows Program Manager screen.

5.4 Combine the Composite Worksheet Form F014, the Composite Report Form F009, and the Internal Deviation From SOP Form F015, in that order, and file the package in the Compositing Notebook.

Prepared by:

Valerie Sadler

_____

Date: _____

Approved by:

Katherine Phillips

_____

Date: _____

Food Analysis Laboratory Control Center (FALCC)
Standard Operating Procedure

6.1.3 TITLE: Gravimetric Determination of Total Lipid in Foods

1. Purpose

To describe the procedure for gravimetric determination of total lipid content in composited, homogenized food samples.

2. Safety

CAUTION: Wear **100% nitrile gloves** at all times when working with chloroform

2.1 Read the material safety data sheet (MSDS) for chloroform.

2.2 Read the material safety data sheet (MSDS) for methanol.

2.3 Make sure a waste bottle for the chloroform and methanol is available in the hood.

2.4 Dispense chloroform in the hood.

3. Materials

3.1 Lipid extract in 500 mL polypropylene centrifuge bottle (SOP #5015)
3.2 100% nitrile gloves (powder free)
3.3 Waste bottle for chloroform, methanol, water, food mixture
3.4 Sartorius Balance (M1-31-3 or M1-22-10) (or equivalent)
3.5 2 ml glass pipet (Fisher # 13-676-26B)
3.6 Sodder iron, 1 mm diameter, 37-50 mm long
3.7 Polypropylene tubing plug for 1/16 ID tubing (Value Plastics, Inc. #PIP210-6)
3.8 Hamilton MicroLab 910 with 25 ml Hamilton gas tight syringe
3.9 N-Evap analytical evaporator (M1-16-3/M1-34-3) with water bath at 60° C
3.10 30-40 ml thick walled glass centrifuge tubes (eg: Corex® #8445-30)
3.11 Metal test tube rack (withstand 121°C)
3.12 Crucible tongs with tygon tubing on tips
3.13 Fume Hood
3.14 Drying Oven at 101°C ± 2°C (M1-8-1) (or equivalent)
3.15 Desiccator with desiccant (M1-39-6) (or equivalent)
3.16 Calibration and Use of Hamilton (SOP# 1003a & 1003b respectively)

3.17 Tweezers
3.18 30 mL Qorpak jar
3.19 Cryogenice marker
3.20 Two, 13.2 x 18 inch teri-wipes

4. Procedure

4.1 Number the 30 mL glass tubes with corresponding sample number and place them in a metal test tube rack.

4.2 Place the rack of tubes in the drying oven (101°C ± 2°C) for 30 min.

4.3 **Read SOP #1003a and b for proper use of the Hamilton MicroLab 910** for syringe preparation and priming procedure and calibration.

4.4 Transfer the rack of glass tubes to the desiccator and allow them to cool for 30 min. The manufacture does not recommended any item above 50°C be placed on the desiccator surface, so the shelf should be covered with two 13.2 x 18 inch teri-wipes.

4.5 Using the tubing tipped crucible tongs, weigh the glass tube and record the weight on the "Total Lipid Worksheet" (Form #F008, copy attached).

4.6 Insert the tubing plug partically into the tip end of a clean 2.0 ml glass pipet (i.e. one pipet per sample). It should be a snug fit, but the plug will not go into the tip entirely.

4.7 Carefully follow "Use of the Hamilton", SOP #1003b, steps 4.1-4.9, to prime the Hamilton with water, methanol, and chloroform.

4.8 Remove the centrifuge bottle from the 22-25°C water bath located in the Gyrotory shaker incubator (step 4.13 in SOP #5015).

4.9 Using a clean 2.0 ml plugged pipet, carefully penetrate the methanol-food layer in the centrifuge bottle with the tip. Once the tip has reached the chloroform layer, insert the sodder iron into the pipet and push the plug out of the tip of the pipet. The pipet tip **must** remain in the **chloroform layer**.

4.10 Carefully follow "Use of the Hamilton", SOP #1003b, steps 4.10-4.25. These steps will provide instructions on pipetting 20 ml of the chloroform layer into a dry, pre-weighed 30 ml glass tube, and explain how to rinse the instrument between samples. (Put SOP #5024 off to the side while pipetting the samples).

4.11 Place the tubes on the N-Evap analytical evaporator. The luer lock needle should be lowered to just inside the glass tube.

4.12 Lower the tubes into a 60°C water bath, and evaporate to dryness under a stream of nitrogen (approx. 60 min.).

Note: Periodically check the tubes during evaporation. The luer lock needles should be lowered to within 4 cm of the solvent level throughout the drying process to ensure optimal drying.

4.13 While the samples evaporate, retrieve the tubing plugs from the centrifuge bottles with a pair of tweezers and place in a Qorpak Jar (or equivalent).

4.14 Rinse the tubing plugs in methanol, then chloroform. Then repeat the rinsing in reverse order to remove any lipids.

4.15 Use water as a final rinse, then allow the plugs to dry.

4.16 Place the evaporated samples into a metal test tube rack and heat them in a 101°C ± 2°C oven for 30 min., cool in a desiccator for an additional 30 min., and weigh to nearest 0.1 mg.

4.17 Record all values on the "Total Lipid Worksheet" (Form # F008)

4.18 Carefully place all waste in the chloroform-methanol waste bottle in the hood.

5. Storage

5.1 The dried sample in glass tube may be covered with foil, and stored at room temperature in the hood (maximum of 24 hr) if time does not allow for oven drying (Step 4.11)

6. Calculations

6.1. Calculate the amount of lipid (extractable material), on a wet weight basis

$$Lipid[g/100g wet weight] = \frac{(W2 - W1) * 80 * 100}{AV * SW}$$

W2 = Weight of glass tube with dried extract (grams)
W1 = Weight of empty dried glass tube (grams)
80 = Total chloroform volume (mls) in the centrifuge bottle
AV = Aliquot volume (mls) dispensed into 30 ml glass tube (i.e. 20 mL)
SW = Sample Weight (grams)

7. Reference(s)

7.1 AOAC 1990. Fats in Foods. Chloroform-Methanol Extraction Method #983.23, In *Official Methods of Analysis*, 15th ed. K. Helfrich (Ed), 1100, Assoc. Off. Anal. Chem. Arlington, Va.

7.2 Bligh E.G. and Dyer W.J., 1959. A Rapid Method of Total Lipid Extraction and Purification. Can. J. Biochem. Physiol. 37:911-917.

Prepared by:

Tina Moler Grove

Date: _____

Approved by:

Katherine Phillips

Date: _____

Food Analysis Laboratory Control Center (FALCC)
Standard Operating Procedure

6.1.4 TITLE: Extraction of Lipids from Foods with Chloroform-Methanol

1. Purpose

To describe the procedure for quantitatively extracting total lipids from *composited* food samples.

2. Safety

CAUTION: Wear **100% nitrile gloves** at all times when working with chloroform

2.1 Read the material safety data sheet (MSDS) for chloroform.

2.2 Read the material safety data sheet (MSDS) for methanol.

2.3 Make sure a waste bottle for the chloroform and methanol is available in the hood.

2.4 Dispense chloroform in the hood.

3. Materials

3.1 Food sample (*composited*) SOP #5005
3.2 Fume Hood
3.3 Stainless steel or Teflon® spatula (9-10 inches long)
3.4 500 mL **silicon coated** (SOP#5008) polypropylenecentrifugebottles (2persample)
3.5 Teflon® tape
3.6 Chloroform - ACS Certified or equivalent
3.7 Methanol - HPLC Certified or equivalent
3.8 0.5 M Sodium Acetate Solution (64.04 g of NaAc/L dH_2O)
3.9 Standard Food Composition Table
3.10 4 Adjustable Repipettors/Dispensers for dispensing $CHCl_3$, MeOH, NaAc & H_2O
3.11 Timer
3.12 100% Nitrile Gloves (powder free)
3.13 25 position orbital shaker by New Brunswick Scientific Co., Inc.(M1-34-4)(or equivalent)
3.14 Beckman J-6B Centrifuge (or equivalent)
3.15 Sartorius Balance (M1-31-3 or M1-22-10) (or equivalent)
3.16 Waste bottle for chloroform and methanol
3.17 Multi-bottle rack to aid in the transportation of centrifuge bottles (Fisher #14-785-1)

3.18 Tub of water equilibrated to 23-25°C in a 25°C Gyrotory shaker incubator (M1-39-11)(or equivalent)

4. Procedure

4.1 Remove samples from 4°C. Place the jars on the lab bench for 20 minutes and allow to come to room temperature.

4.2 Unscrew the lid of the Qorpak jar. Hold the jar at a 30 to 45 degree angle, and stir the diet composite in a circular motion for a minimum of 30 seconds with the rectangular end of a clean, dry stainless steel spatula. While stirring, lift the composite up from the bottom of the jar to the top to insure complete mixing. **Do not lose any of the jar's contents while stirring. If loss occurs, notify supervisor.** Scrape the sides of the jar and scrape off the spatula on the inner edge of the jar. The purpose of this step is to ensure the thawed composite is homogeneous prior to sampling. Proceed **IMMEDIATELY** to step **4.3**.

4.3 Precisely (to the nearest 0.1 mg) weigh 5 g of well mixed sample into a **500 ml siliconized polypropylene centrifuge bottle**. Record the weight and sample number in the "Total Lipid Worksheet" (Form # F008, copy attached)

4.4 Repeat steps **4.2-4.3** once for each sample.

4.5 Based on the moisture content (SOP #5007) of the sample, add enough 0.5 M Na acetate solution so that the total volume of H_2O + Na Acetate equals 32 ml.

Example: 5 g*74.87% = 3.74 ml / 32.0 mL-3.7 mL = 28.3 ml 0.5 M Na acetate

4.6 Add 80 ml of MeOH and 40 ml of $CHCl_3$ to the sample (the ratio of methanol:chloroform:water will be 2:1:0.8 v/v/v).

4.7 Cap the centrifuge bottle and place it on the orbital shaker for **2 hr. at 325 rpm**.

4.8 Add 40 ml of chloroform and shake **(300 rpm)** for an additional **30 min**.

4.9 Switch on Gyrotory shaker incubator, place tub with water (T at about 25°C) in incubator, set control thermostat at 25°C and safety thermostat at 30°C. Check temperature of water bath in the incubator after 30 min., should be 22-25°C.

4.10 Add 40 ml of H_2O and shake **(275 rpm)** for an additional **30 min**.

4.11 Centrifuge at **1473 x g** (2300 rpm in the Beckman J-6B) at temperatures above 4°C but not exceeding 22°C for **10 min** to clarify the bottom layer. Set brake speed at "5".

4.12 Place the bottles into a multi-bottle rack and carefully transport them to the fume hood. Try not to disturbe the layers.

4.13 Place bottles in the 25°C water bath located in the Gyrotory incubator for **15 min**. This will ensure total separation and allow the samples to equilibrate to the set dispensing temperature.

Note: If complete separation does not occur, notify supervisor immediately.

5. Storage

5.1 If necessary, the centrifuge bottle containing the sample can be tightly capped, sealed with Teflon® tape and stored at room temperature for up to 24 hrs. **Extracts must be equilibrated to 25°C before dispensing.**

6. Calculations

6.1 Utilization of chloroform extract: refer to corresponding SOP(s) for gravimetric lipid determination (SOP # 5024), cholesterol (SOP # 5020), and fatty acid methyl esters (SOP # 5010).

7. Reference(s)

7.1 AOAC 1990. Fats in Foods. Chloroform-Methanol Extraction Method #983.23, In *Official Methods of Analysis*, 15th ed. K. Helfrich (Ed), 1100, Assoc. Off. Anal. Chem. Arlington, Va.

7.2 Bligh E.G. and Dyer W.J., 1959. A Rapid Method of Total Lipid Extraction and Purification. Can. J. Biochem. Physiol. 37:911-917.

Prepared by:

Tina Moler Grove

_____

Date: _____

Approved by:

Katherine Phillips

_____

Date: _____

Food Analysis Laboratory Control Center (FALCC)
Standard Operating Procedure

6.1.5 TITLE: Cholesterol Determination in Mixed Diets

1. Purpose

To describe the procedure of quantitation of cholesterol in homogenized composites of mixed diets.

2. Safety

No particular safety precautions have to be taken in addition to those outlined in the various SOPs required for this procedure.

3. Materials

3.1 SOP#5015 - Extraction of Lipids from Food with Chloroform-Methanol
3.2 SOP#5020 - Saponification and Derivatization for Cholesterol Analysis
3.3 SOP#5022 - GC Method for Cholesterol Analysis
3.4 Digital DECpc 433dxLP Computer (M1-29-5)
3.5 Hewlett Packard Laser Jet IIIp (M1-34-7)
3.6 Quattro Pro for Windows® spreadsheet program

4. Procedure

4.1 Extract sterols according to SOP#5015, "Extraction of Lipids from Food with Chloroform-Methanol".

4.2 If new calibration standards or internal standards have to be prepared, proceed with SOP # 5017, if not, continue with **4.3**.

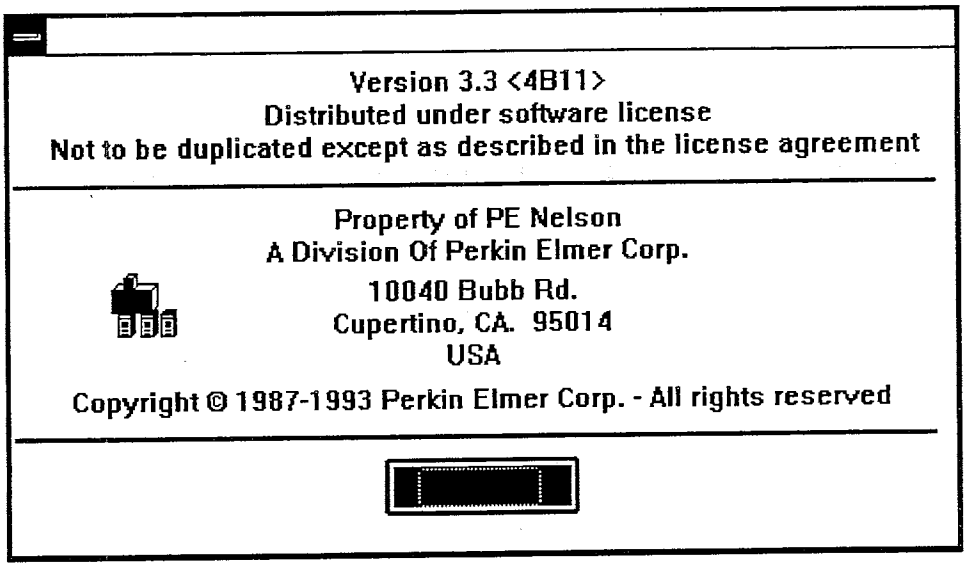
4.3 Saponify and Derivatize cholesterol according to SOP#5020, "Saponification and Derivatization for Cholesterol Analysis".

4.3 Quantify the cholesterol according to SOP#5022, "GC Method for Cholesterol Analysis"

4.4 All data files of GC chromatograms are stored in the form of ASCII files as well as Turbochrom application files. The filename of both, the Turbochrom® data file and the ASCII file is the sample number. However, the Turbochrom® data files have the appendices "RAW" and "RST" for the raw data and the result data file respectively, while the ASCII

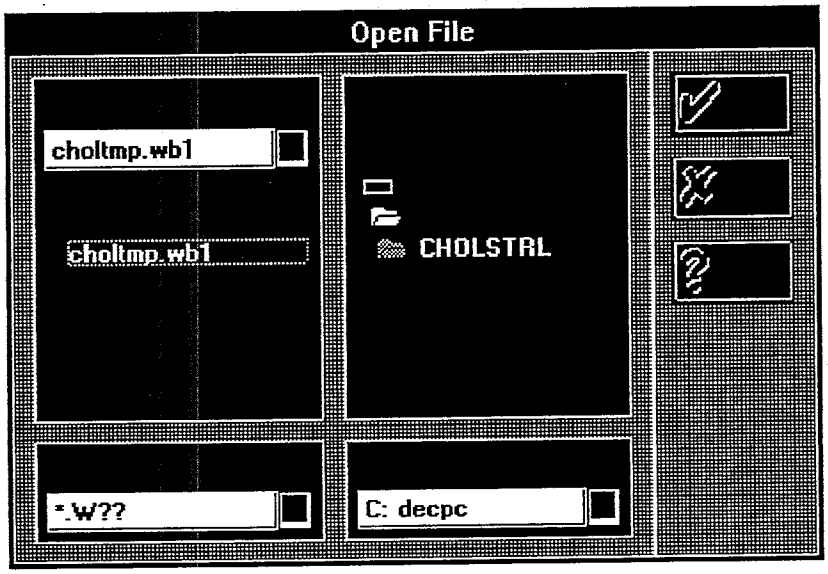
file carries the appendix "TX0" or "TX1". Retrieve the ASCII file with "Quattro Pro for Windows".

4.4.1 Turn on the computer by pressing the ⊘ button on the right-front of the computer. The Microsoft Windows® screen will appear followed by:

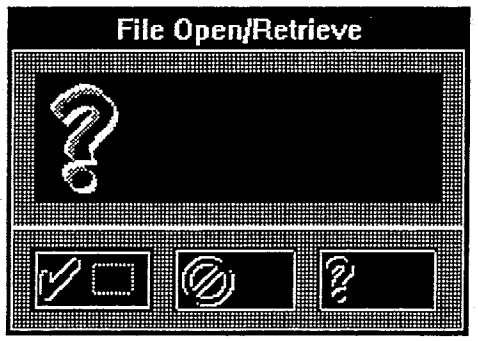


4.4.2 Using the mouse, move the pointer to the *Continue* bar and press the left mouse button. Double-click on the "Quattro Programs" icon and the quattro submenu will open. Double-click on "Quattro Pro for Windows®" and the application will open a blank spreadsheet.

4.4.3 Click on *File*, then *Open*. The following screen will appear:

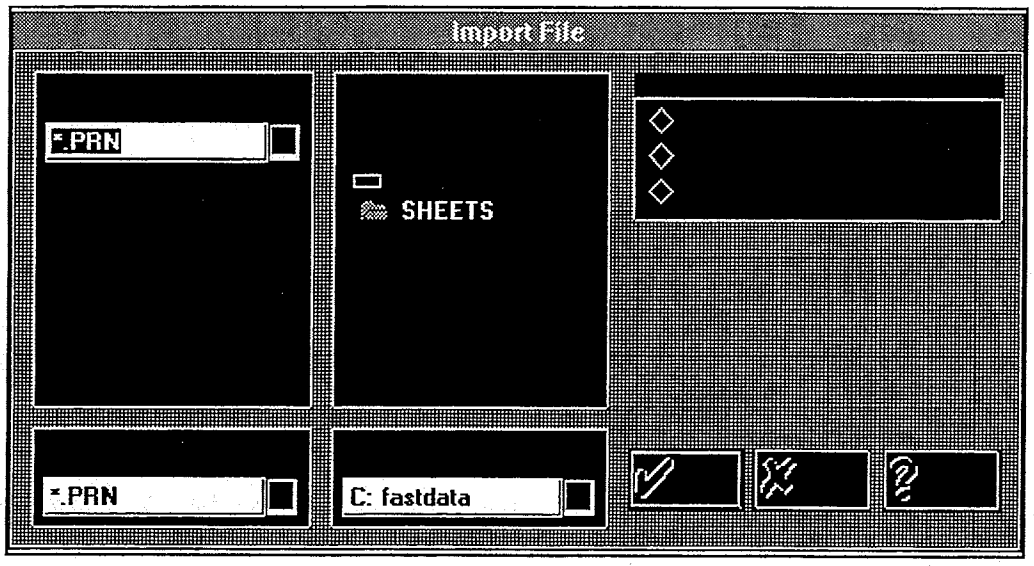


Under *Directories*, double click on *C:*, then *2700*, then *CHOLSTRL*. Under *File Name*, double click on *CHOLTEMP.WB1*, which will bring up the template for the cholesterol quantitation. The following screen will appear;



Click on the *Yes* box. This prevents the template from being overwritten.

4.4.4 Make sure that the highlighted box is in the top-right corner (cell A1). Click on *Tools* and then *Import*, and the following screen will appear:



4.4.5 Type: **.tx?*. Then, using the mouse, click the diamond next to *Comma and "Delimited File* under *Option*, then click on *OK*. All the ASCII files created by Turbochrom® that are in the subdirectory *\cholstrl* will be displayed under *File Name*. Scroll down the list and highlight the file that is to be processed. Then click on the *OK* button. The ASCII file will be retrieved into the spreadsheet.

4.5 To analyze and sort the ASCII file, click on the *Analyze* button in the middle of the spreadsheet. This macro will organize the spreadsheet into a readable format. When the program asks for the weight of the food composite that was used for the lipid extraction, type in the weight, including the decimal point (obtained from total lipid data), and press the ENTER key. When the program asks for the assay number, type in "CL" followed by the assay number (e.g. CL001), and press the ENTER key.

4.6 The *Print Preview* screen will then appear. Click on the button with the blue wrench at the top of the screen. Move the pointer to the beginning of the footer box. Type in the name of

the file followed by .WB1 (i.e. VT301VT.WB1). The entire footer will then look like this: *VT301VT.WB1 / FORM F012 / 19-JUL-93 / page 1 of 1*. Click on the print button (looks like a laser printer with a piece of paper coming out of the top), then click on the *Close* button.

4.7 The report will then print. The final report should look like this:

F012
Date: 19-JUL-93
Revision: New

Cholesterol Analysis Report

Date: 7/15/93 03:33 PM
Analyst: JMC
SOP#: 5026
Assay# CL003
QA\QC _____
Sample Name: Continuous Calibration
Sample Weight: 5.00001 g

CHOLESTEROL ANALYSIS REPORT

Peak #	Component Name	Ret Time [min]	Peak Area [uV-sec]	BL Type	Cholesterol UG	Adjusted Amount	Cholesterol mg/100g wet
5	Cholesterol	22.16	133087.45	BB	41.06	328.51	6.57
6	Dihydrocholesterol	22.45	153068.74	BB	0	0	
			286156.19			328.51	

4.8 When the spreadsheet comes back onto the screen the prompt in the top-left corner will read, "Press ENTER to erase the Macro and continue..." Press the ENTER key. This erases the *Analyze* program from the spreadsheet, which saves disk space.

4.9 To save the spreadsheet, click on *File*, then *Save As...* Type the file name followed by .WB1 (e.g. VT301VT.WB1). Click on *File*, then *Close*.

4.10 Repeat steps **4.4.3** through **4.9** for each sample in the assay.

4.11 When all of the worksheets have been completed, obtain the sample information from QA/QC, and make out the final report.

4.11.1 In Quattro Pro for Windows®, click on *File* then *Open*. Highlight the file *C:\2700\CHOLSTRL\F025.WB1*.

4.11.2 Type in the dates, the analyst's initials, the assay#, and the %moisture. Fill in the rest of the spreadsheet with the data from the worksheets. Each composite that is run in one assay will have two sample numbers. Assign the sample numbers, that have the same sample description, to the columns *Sample #1* and *Sample #2*. The Cholesterol (mg/100g wet) for sample #1 should be listed under column 1, likewise the Cholesterol (mg/100g wet) for sample #2 should be listed under column 2. The computer will calculate the mean of the two and the Cholesterol (mg/100g dry).

4.11.3 Click on *File* then *Print*. Click on the *Preview* box. The document will then be presented as it will appear on the page. Click on the blue wrench button at the top of the screen, move the pointer to the beginning of the footer text box, and click the left mouse button. Type in the assay number followed by .WB1 (i.e. CL001.WB1). The footer should then look like this:

CL001.WB1 / FORM F025 / 26-JUL-93 / page 1 of 1

Change only the assay number in the footer. The date in the footer is the date that the form format was approved. Click on the *OK* button.

4.11.4 Click on the *Print* button then the *Close* button. Press the *Home* key on the keyboard, and save the file by clicking on *File, Save As...* Type in the assay number followed by .WB1 (i.e. CL001.WB1), then click on *OK*.

4.12 The assay request form, worksheets, and final report should be turned in to the QA/QC Officer, for processing.

5. References

Figures were obtained from Turbochrom® 3 and Quattro Pro for Windows® software.

Prepared by:

James Cooke

_____

Date: _____

Approved by:

Katherine Phillips

_____

Date: _____

6.1.6 DETERMINATION OF MOISTURE BY MICROWAVE DRYING

In the process of being developed.

Food Analysis Laboratory Control Center (FALCC)
Standard Operating Procedure

6.1.7 TITLE: Determination of Moisture in Diet Composites by Vacuum Oven Drying

1. Purpose

To describe the procedure for determining the moisture content of composited mixed diets by drying in a vacuum.

2. Safety

CAUTION:

2.1 Care should be taken when handling hot pans.

3. Materials

3.1 aluminum pans with tightly fitting lids (catalog #08-722 Fisher Scientific or equivalent)
3.2 diet composite (SOP# 5005)
3.3 Use and handling of food composites samples (SOP# 5028)
3.4 Sartorius Basic analytical balance (M1-22-10) (SOP# 1008)
3.5 Fisher Isotemp® vacuum oven (M1-30-6) (SOP# 1007)
3.6 Precision™ vacuum pump (M1-10-1) (SOP# 1007)
3.7 Desiccator containing desiccant, e.g. Drierite® (anhydrous calcium sulfate) (catalog #12-890 Fisher Scientific or equivalent.)
3.8 long-handled forceps or other tool to remove samples from oven
3.9 Stainless steel spatula

4. Procedure

NOTE: Do not handle pans with bare hands; use clean forceps, or handle using a Kimwipe®.

4.1 Thaw food composite samples from -60 degrees according to SOP# 5028

4.2 Place clean, dry pans (uncovered) and lids (1 each per aliquot to be analyzed) in vacuum oven (SOP# 1007) at 65 - 70°C and a vacuum pressure of 25 inches Hg for 1 hour; ensure pans rest on a clean surface. If several pans are used, it is convenient to place them on an aluminum foil tray which has been perforated to allow air circulation.

4.3 Lightly cover pans with lids, immediately transfer to desiccator, and allow to cool completely (at least 15 minutes - it is O.K. to wait longer if convenient).

4.4 Accurately weigh pan and lid on analytical balance (SOP# 1008); record weight.

4.5 For each sample to be assayed:

4.4.1 Unscrew the lid of the Qorpak jar. Hold the jar at a 30 to 45 degree angle, and stir the diet composite in a circular motion for a minimum of 30 seconds with the rectangular end of a clean, dry stainless steel spatula. While stirring, lift the composite up from the bottom of the jar to the top to insure complete mixing. Do not lose any of the jar's contents while stirring. If loss occurs, notify supervisor. Scrape the sides of the jar and scrape off the spatula on the inner edge of the jar. (The purpose of this step is to ensure that the thawed composite is homogeneous prior to sampling - critical). **Immediately proceed to step 4.4.2**

4.4.2 **Immediately** add approximately two (2) grams of the composite to the pan on the balance using a stainless steel spatula; record weight.

4.4.3 If more than one aliquot is taken from the jar, repeat step 4.4.1- 4.4.2 for each aliquot.

4.6 Place pans (uncovered) and lids in vacuum oven (65-70°C; 25mm Hg) for three and a half (3.5) hours **with the vacuum pump running**. Time begins when oven temperature reaches 65^0 C.

4.7 Slowly release the pressure in the oven (SOP# 1007). Using tongs cover each pan tightly with its corresponding lid while still in the oven, then immediately transfer to the desiccator.

4.8 Cool to room temperature (ca. 15 minutes). Weigh and record weight to nearest 0.0001g.

5. Results

5.1 Calculation of moisture content

A = weight pan and lid (grams)
B = weight pan, lid, sample (undried; grams)
C = weight pan, lid, sample (dried; grams)

$$\% \text{ moisture} = \frac{(B-C)*100}{B-A}$$

6. Reference

Association of Official Analytical Chemists, Official Methods of Analysis, 1990 (15th edition); Method 934.01, p. 69; modified

Prepared by:	**Approved by:**
Pam Crvich	Katherine Phillips
Date: August 24, 1993	Date: _____

Food Analysis Laboratory Control Center (FALCC)
Standard Operating Procedure

6.1.8 TITLE: GC Method for Fatty Acid Methyl Ester Analysis

1. Purpose

To describe the procedure for programming the GC using Turbochom 3, and the GC control panel.

2. Safety

CAUTION: The Gas Chromatograph uses highly pressurized Hydrogen as a fuel source for the flame ionization detector (FID). Hydrogen, when mixed with air is highly explosive, make sure that the Hydrogen is turned off when the flame is not lit.

2.1 Read the material Safety Data Sheet (MSDS) for iso-octane.

2.2 Do not touch the injector, it is extremely hot.

3. Materials

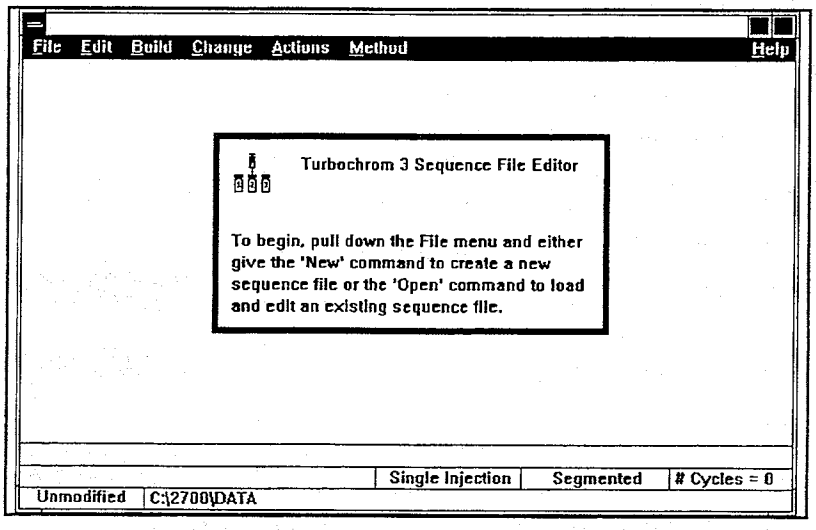
3.1 Gas Chromatograph - Perkin Elmer Autosystem GC with Autosampler (Instrument B, M1-34-5) (Column: Stabilwax®, 30 m, 0.25 mm ID, 0.1 μm df - Restek # 10608)
3.2 Digital DECpc 433dxLP Computer (M1-29-5)
3.3 Turbochom 3 software system for data aquisition and analysis
3.4 Hewlett Packard Laser Jet IIIp
3.5 Fatty acid methyl ester samples (see SOP# 5010), blank - iso-octane, continuous calibration standard - AOCS Rapeseed Oil Reference Mix (Matreya Catalog # 1083)

4. Procedure

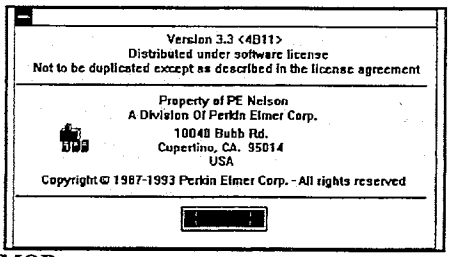
4.1 Turn on the computer by pressing the ⊘ button on the right-front of the computer. The Microsoft Windows® screen will appear followed by:

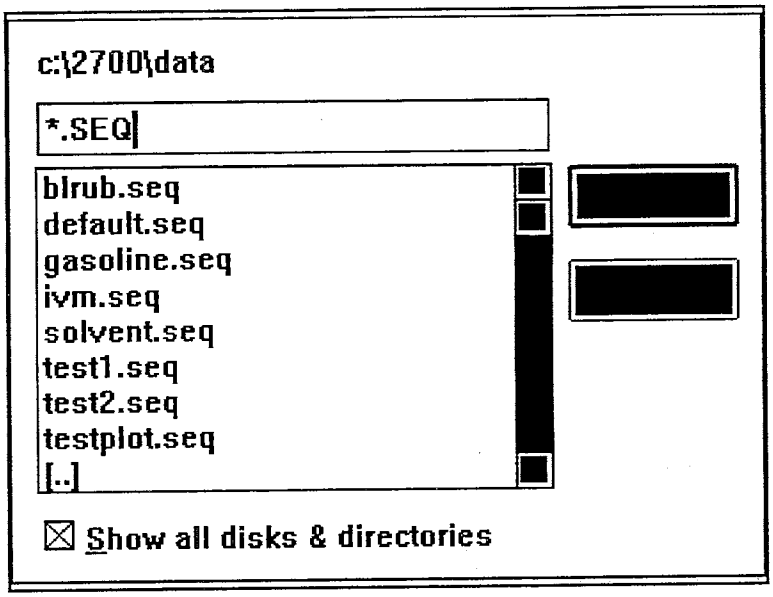
4.2 Using the mouse, move the pointer to the *Continue* bar and press the left mouse button. The Turbochom 3 Main Menu Bar will appear at the bottom of the screen.

4.3 Move the pointer to *Editors* and press the left mouse button. Click on *Sequence*, and the *sequence editor* will appear.



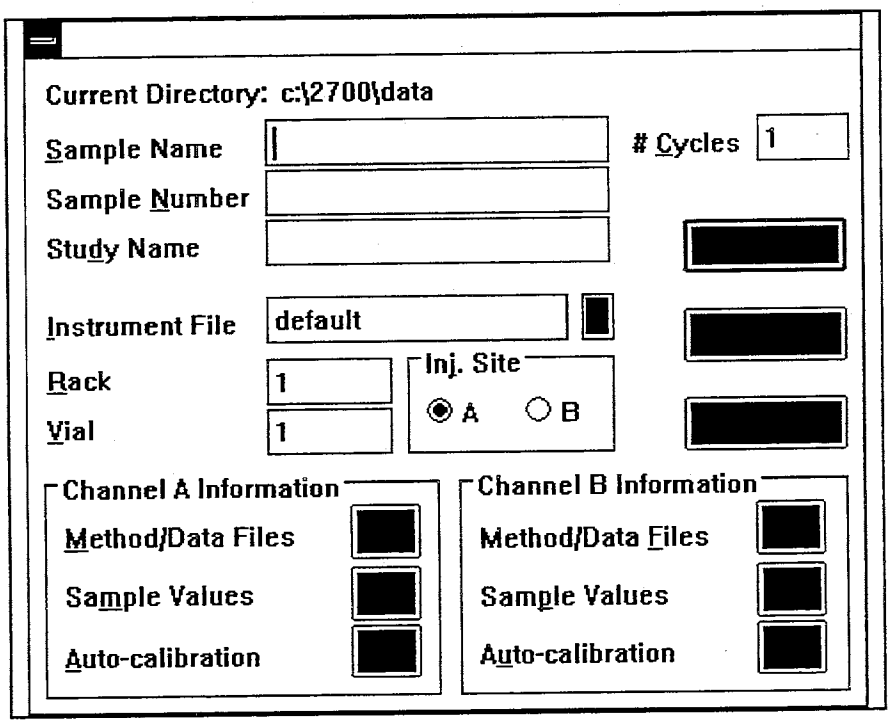
4.4 Click on *File*, then *Open*, and the following screen appears:





Double click on *[..]*, then on *[fame]*. Highlight *default.seq* by clicking on it and then click on *OK*. The editor loads the default sequence file and returns to the *sequence editor* (**4.3**) screen.

4.5 Click on *Build*, then on *Append New Cycles*, and the *Add Sequence Information* Screen will appear:



The cursor blinks in the *Sample Name* box. Type in the name of the sample for the first sample in the batch (e.g. VT301VT). The *Sample Number* box should read 3. Move the cursor to the *Study Name* box, click and type "FAMEASSAY" followed by the the assay number as assigned (e.g. FAMEASSAY001). Move to the *Vial* box and type in the number that the vial will be placed under on the autosampler rack (since

slots 1 and 2 are reserved for a blank (isooctane) and a continuous calibration sample, 3 is the first available slot).

4.6 Click on the *Add* box. The Sample Number will then change to 4.

4.7 Type in the sample name for the next sample in the batch, and change the *vial number* to 4 or the next consecutive number. Click the *Add* box.

Note: While the sample numbers will change automatically, the sample names and vial numbers have to be changed manually.

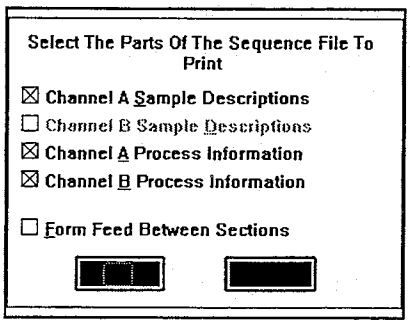
4.8 Repeat **4.7** for all the samples in the run. Click on the *Quit* box to exit the sequence editor after the last sample has been added.

4.9 It is necessary to edit the sequence in the sequence spreadsheet. To view the spreadsheet, click on *Edit* in the *Sequence Editor* Screen, then *Channel A*. The spreadsheet can be viewed and edited using the mouse or arrow keys. Change all of the entries in the *DATA* column to the path followed by the name of the sample in that row (e.g. c:\2700\fame\VT301VT). The entries in the *DATA* column for the blank and the continuing calibration (Con-Cal) samples should be BLNK and CCAL respectively followed by the assay number as assigned (e.g. c:\2700\fame\BLNK001). Check all other entries for the correct information that were just entered for all the samples.

4.10 After editing the spreadsheet, click the *minus sign* box in the left corner above the O of *Options* and click on *Close*.

4.11 Save the file by clicking *File*, then *Save As...* **(Caution: Do not use the *save* command since it will overwrite the default file)** Type in the assay number (as assigned, e.g. FA001) as the filename of the sequence, including the correct path (the path will always be **C:\2700\fame**\FA#.seq). Click on *OK*. (In case of a warning screen, do not overwrite the old sequence file! Check at once with a supervisor on the correct assay number.)

4.12 Print the sequence by clicking on *File*, then *Print*. The following screen will appear:



Since only the box labeled, *"Channel A Sample Description,"* and *"Channel A process Info"* should be marked by an X, click the other boxes so that the X disappears. Then click the *OK* box. Wait for the printout. Cut and paste the sequence printout into the GC-B Log Book.

4.13 Exit the sequence editor by clicking on the *minus sign* box in the top left-hand corner of the screen then click *Close*.

4.14 On the GC control panel press *DET TEMP* (white button), then punch in 200 (black buttons), and press *ENTER* (yellow button). This will allow the detector block to heat up for lighting the detector.

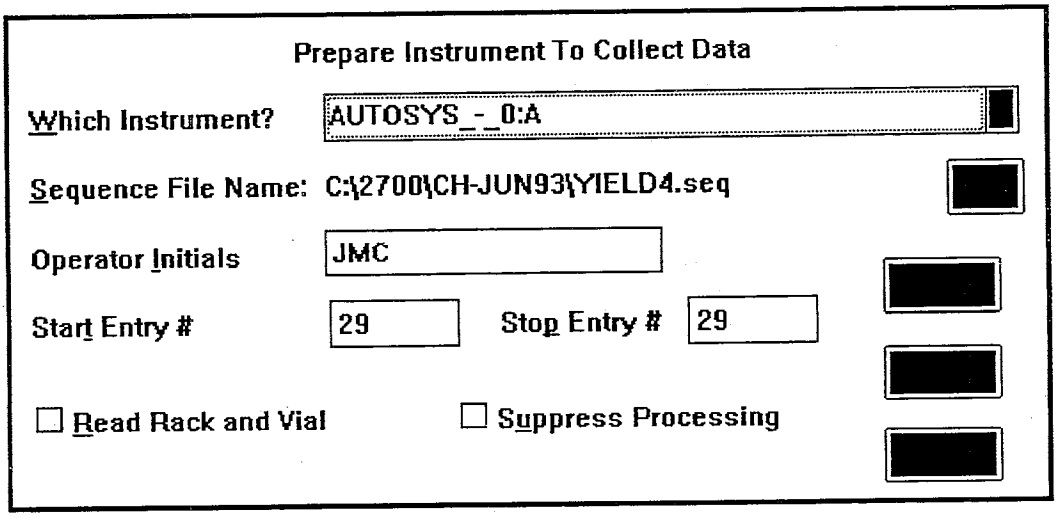
4.15 Remove the blank (isooctane), the continuous calibration standard and the samples to be run (SOP# 5010), from the freezer, and place them in their respective vial holders in the GC as determined by the sequence file.

4.16 Open the hydrogen and air gas tanks designated for this GC (instrument B). On the GC, open the split, air and hydrogen valves by turning the valve knobs (towards the rear end) counter-clockwise to "ON". Check the carrier gas pressure (19.9-20.1 psi) by pressing the *carrier gas* (white) button on the front panel.

4.17 Press *Auto Zero* (white) and light the Flame Ionization Detector by opening the lid on the right top of the GC, removing the ignitor, closing the air valve, pressing the ignitor on top of the detector, and slowly opening the air valve. A popping sound should be heard, and the value on the front panel should jump to several mV. The value should then drop down to 0.5 to 1 mV, and remain there. Open the air valve completely. If the value drops below 0.05 mV, the detector failed to ignite and the procedure has to be repeated.

4.18 Make sure that wash vials 1 and 2 (in the center of the autosampler tray) are filled with iso-octane, and that waste vials 1 and 2 are empty.

4.19 From the Turbochrom 3 main menu bar (**4.2**), click on *Acquire*, then click *Download*. The following screen will appear:



Highlight *AUTOSYS_-_0;B* by clicking on the ↓ key. Click on the ⇒ button next to the Sequence File Name, double click on *[fame]* and highlight the sequence file that was created in steps **4.3** through **4.11**. Click on *OK*. Type in the Operator Initials, and click on the *Verify* button. The sequence will then be dowloaded to the GC. From the *Data Aquisition* screen, click *Inst*, then *Start*.

4.20 Make sure the printer is turned on and paper is loaded into the paper tray.

4.21 SHUTDOWN procedure: After the last sample run has been completed, in the *Data Aquisition* Screen of the *Aquire* menu click on *Comm* (for Communication). Click *Release Control*, highlight *AUTOSYS_-_0;B* and click OK. Then click on the *minus sign* box in the top left-hand corner of the screen and click *Close*. On the GC, turn off the split, air and hydrogen knob valves, and turn off the air and hydrogen tanks (**not the Helium tank!**). On the GC control panel, press the *DET TEMP* button and press *OFF* (black button) then *Enter*. Then press *INJ TEMP,* type in 100 and *Enter*, then press *OVEN TEMP*, punch in 50 and press *ENTER*.. The GC is now shut down and the computer and printer can be turned off as soon as all chromatograms are printed.

4.22 Remove the caps of the crimp-seal vials using the decapper. Reseal the vials with new caps and mark the level of the liquid in the vial. Store the vials in a freezer.

5. Calculations

5.1 The results consist of a chromatogram and a report and will be printed automatically by the computer. The *Adjusted Amounts* are μg of fatty acid methyl esters per sample. In addition, the program generates an ASCII file that will be used for further quantitation (SOP#5025). Punch holes in the reports and place them in the fatty acid data notebooks.

6. Reference(s)

6.1 PE Nelson Turbochrom 3 User's Guide 1992

6.2 All figures were obtained directly from the Turbochrome 3 software (version 3.3)

Prepared by:

Ingolf Gruen

_____

Date: _____

Approved by:

Katherine Phillipis

_____

Date:_____

CLASSIFICATION OF FATTY ACIDS

SFA	MUFA	PUFA	omega 3-FA	other
10:0	14:1	18:2*	18:3n-3 (α-linolenic)	Other FA
11:0	16:1	20:2	20:5n-3 (EPA)	Unidentified peaks
12:0*	17:1	20:3	22:6n-3 (DHA)	
13:0	18:1*	20:4		
14:0*	19:1	22:6		
15:0	20:1			
16:0*	22:1			
17:0				
18:0*				
20:0				
22:0				
24:0				

* Individual concentrations of these fatty acids will also be reported (as TAGs). The values will be included in total SFA, PUFA, MUFA as well.

Food Analysis Laboratory Control Center (FALCC)
Standard Operating Procedure

6.1.9 TITLE: Determination of Ash in Diet Composites

Scope: This procedure applies to diet composites with 60 - 80% moisture content.

1. Purpose

To describe the procedure for determining the ash content of diet composites.

2. Safety

2.1 Wear powder free gloves at all times.

2.2 Use caution when operating furnace at 550°C.

3. Materials

3.1 porcelain crucibles (Coors# 60105, Fisher#07965-D, or equivalent)
3.2 heat-resistant ink pen
3.3 powder free gloves
3.4 long-handled metal forceps
3.5 desiccator
3.6 analytical balance (e.g. Sartorius Basic, M1-31-3, SOP# 1008), or equivalent
3.7 diet composite (SOP# 5005); (if at -60°C, thawed in refrigerator for 12-48 hours)
3.8 stainless steel spatula
3.9 Fisher Isotemp Vacuum Oven (M1-30-6, SOP#1007), or equivalent
3.10 Precision Vacuum Pump (M1-10-1, SOP#1007), or equivalent
3.11 muffle furnace (550°C), Dr. Bunce-Room 201
3.12 I.B.M. personal computer and printer (e.g. M1-34-10), or equivalent

4. Procedure

Note: To avoid weight from fingerprints, do not handle the crucibles with bare hands; use forceps or wear powder free gloves. Use forceps when removing crucibles from the furnace and the vacuum oven.

Note: Record all data on an "Ash Worksheet" (see enclosed sheet-Form F007).

Note: Before beginning the analysis, select an assay number from the assay number log-out book. See note in front of book for instructions.

4.1 If not already labelled, label each crucible with a heat-resistant ink pen for identification.

4.2 Place empty crucibles in the muffle furnace. Close the muffle furnace door.

4.3 Turn the muffle furnace switch to "on". Turn the temperature dial to 550°C (once the muffle furnace temperature reaches 550°C, the cycle light will begin to flicker).

4.4 Leave the empty crucibles in the muffle furnace at least 12 hours (e.g. overnight), timed from when the temperature reaches 550°C (takes ~ 1 hour to reach 550°C).

4.5 Turn the muffle furnace switch to "off" and crack the door.

4.6 Allow the crucibles to cool in the muffle furnace for 30 minutes.

4.7 Place the crucibles in a desiccator and allow to cool completely (~1 hour).

Note: Crucibles may be pre-ashed (steps **4.2-4.7**) and stored in a desiccator until needed.

4.8 Turn on the balance. The display should read "0.00000g".

4.9 Turn on the computer. Turn on the monitor. The display should read "C:\>".

4.10 At the "C:\>" prompt, type "cd\quat". Press "Enter". At the "C:\QUAT>" prompt, type "q". Press "Enter".

4.11 When the quattro screen appears, hit the "/" key to select the quattro menu.

4.12 Move the cursor bar to "file" and press "Enter" to select the file menu.

4.13 Press "Enter" a second time to retrieve the list of files. Move the cursor bar to "Ash.wk1" and press "Enter" to open the file.

4.14 Weigh a cooled, pre-ashed crucible to the nearest 0.0001 gram.

4.15 Record the crucible weight and the crucible number in the spreadsheet. To record the crucible weight, press the "print" key on the balance to transfer the weight value to the appropriate box in the spreadsheet.

4.16 After recording the crucible weight, press "tare" to zero out the crucible weight.

4.17 Unscrew the lid of the Qorpak jar. Hold the jar at a 30 to 45 degree angle, and stir the diet composite in a circular motion for a minimum of 30 seconds with the rectangular end of a clean, dry stainless steel spatula. While stirring, lift the

composite up from the bottom of the jar to the top to insure complete mixing. **Do not lose any of the jar's contents while stirring. If loss occurs, notify supervisor** and fill out F015 Internal Deviation form. Scrape the sides of the jar and scrape off the spatula on the inner edge of the jar. The purpose of this step is to ensure the thawed composite is homogeneous prior to sampling. Proceed immediately to step **4.18**.

4.18 Using the rounded end of a stainless steel spatula, weigh 2 to 3 grams of the diet composite (to the nearest 0.0001 g) into the crucible.

4.19 Record the sample weight and the sample number in the spreadsheet. To record the sample weight, press the "print" key on the balance to transfer the weight value to the appropriate box in the spreadsheet.

Note: The total weight (crucible and sample weight to the nearest 0.0001g) is already calculated in the spreadsheet.

4.20 Repeat steps **4.14-4.19** for each sample.

4.21 Place the crucibles in the vacuum oven, uncovered, for 6 hours at a temperature of 65 to 70°C and at a pressure of 25 inches Hg (see SOP #1007 for operating instructions).

4.22 Place the crucibles in a desiccator to transfer to muffle furnace.

4.23 Place the crucibles in the muffle furnace. Close the muffle furnace door.

4.24 Turn the muffle furnace switch to "on". Turn the temperature dial to 550°C (once the muffle furnace temperature reaches 550°C, the cycle light will begin to flicker).

4.25 Leave the crucibles in the muffle furnace at least 12 hours (e.g. overnight), timed from when the temperature reaches 550°C (takes ~ 1 hour to reach 550°C). Make a note in bench book of exact heating time.

4.26 Turn the muffle furnace switch to "off" and crack the door.

4.27 Allow the crucibles to cool in the muffle furnace for 30 minutes.

4.28 Place the crucibles in a desiccator and allow to cool completely (~ 1 hour).

Note: A grayish white powder should result after ashing. If not, notify supervisor and fill out form F015-Internal Deviation form.

4.29 Weigh each crucible with ash residue to the nearest 0.0001 gram.

4.30 Record the weight in the spreadsheet. To record the weight, press the "print" key on the balance to transfer the weight value to the appropriate box in the spreadsheet.

4.31 Once all the data has been obtained, save the data program with a filename that corresponds to the assay number (i.e. assay # A1 has the filename A001.

5. Storage

5.1 Discard the ash residue after weighing.

6. Calculations

6.1 Calculation of percent ash on wet basis

A = weight of crucible, ash (grams) (step **4.21**)
B = weight of crucible, (pre-ashed; grams) (step **4.8**)
C = weight of crucible & food sample (undried;grams) (step **4.11**)

$$\% \text{ ash (wet)} = \frac{(A - B)}{(C - B)} * 100$$

Report ash as grams/100 grams wet weight.

Note: The calculations for percent ash on a wet basis are performed in the spreadsheet.

7. Reference

7.1 AOAC Official Methods of Analysis, "Ash of Sugar and Sirups Final Action", #31.012 and #31.013, 1984.

Prepared by:

Kristen Lekstrom

_____

Date: _____

Approved by:

Katherine Phillips

_____

Date: _____

CHAPTER 7

DATA MANAGEMENT

TABLE OF CONTENTS

7.1 GENERAL INSTRUCTIONS FOR FILLING OUT FORMS

7.1.1 Background

The Dietary Effects on Lipoprotein and Thrombogenic Activity (DELTA) study will use paper forms to collect most data. Data from these paper forms will then be entered at a later date either into a computer-assisted data management system (DMS) located at each field center and the food analysis laboratory or into non-standard format files at the central lipid and hematology laboratories.

The purpose of this section is to provide instructions for completing these paper forms. It should be read carefully prior to working with any forms. The form-specific instructions should then be read before filling out each form.

7.1.2 Form Structure

Most paper forms in DELTA are similar in design to the forms layout of the computer screens used for data entry. The paper forms are organized by pages, but the forms in the data management system are organized by computer screens. This may cause some problems in locating an item on a paper form and the corresponding item on the computer screen, however, the items are listed in the same order.

The forms are structured with a header of the form title, form code, form version number, and date of form version across the top of each page. The first page begins with the key data fields of participant DELTA ID number and a visit date. The form code, form version number, participant DELTA ID number, and visit date comprise a unique set of data which identify an individual form type for a participant at a certain point in time on the study database.

Following these header and key data items on the forms are the questions for the participants. Instructions to the interviewer are in brackets ("[]") on most forms.

An example of a typical "first page" is presented in Figure 1.

Figure 1: Example of DELTA Form - First Page

DELTA

Form Code: PWM Version: A 7/06/93

Participant Weekly Monitoring Form

DELTA ID: _____ 1. Monday's Date: _____ (mm/dd/yy) 2. Personnel Code Number: ____

WEIGHT

[Participants are weighed before dinner, without shoes or coats.]

3. a. Date of first weekly weight: _____ (mm/dd/yy)

First weekly weight, either in lbs or kg: b. lbs: _____ or c. kg: _____

d. Current calorie level: _____

4. a. Date of second weekly weight: _____ (mm/dd/yy)

Second weekly weight, either in lbs or kg: b. lbs: _____ or c. kg: _____

d. Current calorie level: _____

EXERCISE

[Exercise is recorded at the first weekly visit following the weekend.]

5. a. In the past week, has your exercise level changed? YES NO

b. If YES, how has your exercise level changed: [Circle letter preceding your selection]

A...More active
B...Less active
C...No exercise

ILLNESS

6. Have you been ill in the last week? YES NO [If NO, skip to question 9]

If YES, describe illness: _____

7. a. Did you take any medications for your illness? YES NO

If YES, specify the name of the medication and amount of medication:

b. Medication: _____ c. Total weekly amount: _____

d. Medication: _____ e. Total weekly amount: _____

f. Medication: _____ g. Total weekly amount: _____

7.1.3 General Instructions for Completing and Correcting Items on the Forms

All items fall into three main categories: (1) fill in the space, (2) multiple choice, and (3) narrative description. Techniques for completing each of these types of items, as well as making corrections, are described below. A general rule is to record information only in the spaces provided (except for some error corrections).

7.1.3.1 Fill in the Space: Recording Information

This category is similar to filling in boxes with numeric or alphabetic information of a pre-specified length and within a range of valid values. Examples include participant's last name, date of birth, or sitting blood pressure measurements. The maximum length of the field is not apparent on the paper form, however, a maximum field length is indicated on the computer screens. The instructions below correspond to the entry in the data management system.

When alphabetic information is required, enter the response beginning in the leftmost space using capital letters. Punctuation may be included.

Example: If the participant's last name were O'Reilly, it should be entered as follows:

Last Name: O'REILLY___

If the response contain more characters than there are spaces, begin with the first character and enter as many characters as there are spaces.

Example: If the subject's last name were Hobgoodnotting, it should be entered as follows:

Last Name: HOBGOODNOTTI

Whenever numerical responses are required, enter the number so that the last digit appears in the rightmost space. Enter leading zeroes where necessary to fill all spaces. (This does not apply to the address section or to any item which combines alphabetic and numeric information. Such items should be treated as alphabetic.)

Example: If the participant's diastolic blood pressure were 96, it should be entered as:

Diastolic: 096

When dates are recorded, slashes ("/") are required as the separator characters for month, day, and year. The format to be used to record dates is indicated under the spaces as month/day/year. Use leading zeros within each date unit (month or day or year) so that each space is filled.

Example: Data collected on April 3, 1993 would be entered as:

Today's Date: 04/03/93

DELTA usually records time using a 12-hour clock, with AM or PM indicated separately. Colons (":") are used as the separator character for hours and minutes. The format to be used is indicated under the space. Use leading zeros within each time unit (hour or minute) so that each space is filled. Note that midnight is recorded as 12:00 AM, and noon is recorded as 12:00 PM.

Example: A time of fasting determination of 8:05 in the morning is entered as:

a. Time: 08:05 b. AM (A) or PM (P): A

7.1.3.2 Fill in the Spaces: Correcting Mistakes

If a number or letter is entered incorrectly on the paper form, mark through the incorrect entry with an "X". Code the correct entry clearly above the original incorrect entry. Record the initials of the person correcting the mistake and the date of correction next to the correction.

For example, if the participant's systolic blood pressure were actually 130, but was incorrectly recorded as 139, put an "X" through the 9 and write a 0 above the correction. Place your initials and date of correction beside the corrected entry.

If a mistake is made, corrected, and then it is discovered that the correction is incorrect (the value in the example above should be 132), make a second correction by placing an "X" through the handwritten 0 and writing a 2 beside the correction. Again, place your initials and date of correction beside the corrected entry.

7.1.3.3 Fill in the Spaces: Unknown or Inapplicable Information

If an item of this type (alphabetic or numeric) *does not apply* to this participant, leave the space blank. For example, if the participant does not have a work phone number, that item is left blank.

If the item *does apply*, but the response is unknown, mark through the space with *two* horizontal lines (equal signs "=").

For example, the participant was asked date of birth but does not recall the month and day. The question does apply because it can verify the age of the participant for stratification by age in data analyses. In this case, the response would look like "==/==/40". The same response would be entered on the computer screen.

7.1.3.4 Multiple Choice: Recording Information

In this type of question several alternatives are given for the answer, each having a corresponding letter or number. When it is decided which alternative is most appropriate, circle the corresponding letter in the space provided. Always circle *one letter only* unless otherwise specified.

Example: How many years has it been since your last cigarette?
A Less than 1 year
B 1 year or more

In the above example, for a response of 1 year or more, circle response B. Enter B as the response on the computer screen.

For questions with YES/NO responses, circle either YES or NO.

Example: Do you now smoke cigarettes? YES NO

In the above example, if the participant does not now smoke cigarettes, circle response NO. Enter N for NO as the response on the computer screen.

7.1.3.5 Multiple Choice: Correcting Mistakes

If a response is coded incorrectly, mark through the incorrectly coded response with an "X" and circle the correct response.

Example: How many years has it been since your last cigarette?
A Less than 1 year
B 1 year or more

The actual response is A, but B was circled incorrectly. Put an "X" through the circled response B and circle the corrected response A. Place your initials and date of correction next to the corrected entry.

If a mistake is made, corrected, and then it is discovered that the correction is incorrect (correct response is actually B in the example above), make a second correction by placing an "X" through the circled response A and writing and circling the corrected response B. Place your initials and date of correction next to the corrected entry.

To record the correct response on the computer screen of the data management system, enter the correct response in the space provided.

7.1.3.6 Narrative Description

On forms where narrative descriptions or comments are requested, space is provided to write this information. The person who made the hand-written comments should review the descriptions for accuracy and legibility. Abbreviations should be avoided. These narrative descriptions are

entered in fixed length fields or open-ended note logs in the data management system (see instructions on the data management system for details).

Responses to fixed length items on the form, such as medication name or type of physical activity, is entered in BLOCK CAPITAL LETTERS in the data management system.

7.1.4 Completing Key Data Information

The following guidelines should be observed in completing the key data information following the "header" located at the top of the first page on all forms:

7.1.4.1 DELTA ID: Apply the pre-printed ID label, or write in the participant's 5-character ID number. Column 1 contains a letter identifying the field center, column 2 contains the current protocol number 1, and columns 3-5 contain a 3-digit number from 001-999 uniquely identifying each participant.

The letters for each field center are as follows:

C	Columbia
L	Louisiana
M	Minnesota
P	Penn State

7.1.4.2 Today's Date: Fill in the date of the participant's visit to the field center. Enter the date as month/day/year using zeros to fill in all spaces. The participant's visit date is also referred to as Monday's Date on the Participant Weekly Monitoring Form and as Date Blood Drawn on the Lipid Profile Forms.

7.1.5 Skip Patterns ("Go to" Spaces)

Skip patterns occur in many multiple choice type items. Here, if a certain response is selected, it is necessary to skip over one or more items to the next applicable item. This is indicated by a "go to" instructions on the form. If the response is selected which necessitates a skip, the next item to be asked is indicated in the instructions on the form. If the other response is selected, always proceed to the next item unless otherwise directed.

Occasionally, a skip pattern will occur in a fill-in type item. In those instances, specific instructions are provided on the form. Again, if the skip criteria are not satisfied, continue with the next item.

7.1.6 Security of Data

DELTA paper forms contain confidential participant name and information and are to be stored in a secured filing cabinet or room. The file cabinet or room containing the paper forms should be a reasonable distance from the computer system and diskettes to minimize loss of data in case of a disaster.

Only personnel who are authorized to enter data on the computerized data management system should be given the Access Code and Password for the DMS to prevent unauthorized entry. If unauthorized persons have obtained the Access Code or Password, change these using the procedures described in the instructions for using the data management system.

Backup copies of all data files and diskettes should be maintained to avoid loss of data. The work and backup diskettes should be stored in a secured location, as far as conveniently feasible from the microcomputer, to reduce the risk of data loss.

The Coordinating Center can restore data that has been received, but the centers must ensure that at least one of their copies of currently entered data (paper forms, files on diskettes or the computer system) will survive an occurrence of data loss such as computer theft, hard disk crash, automatic sprinklers, or coffee spills.

7.2 SET-UP OF THE DMS

7.2.1 Installation

The computers are shipped to the field centers with the DELTA data management system software installed. In addition, word processing, electronic mail, and utility programs are installed for your use on the DELTA Study. Instructions to set up the computer and user's guides for the software are shipped with the computers.

During the study, it is possible that the CSCC may release an upgrade to the DMS due to a form version change, a new feature or report, or a problem fix. Instructions to install the upgrade will be distributed with the software upgrade.

Important notice: If you plan to install other software on the computer, please notify the Coordinating Center prior to your installation. Some software could cause interference with the functioning of the DMS.

7.2.2 Preparing Diskettes and Backup Tapes

Refer to section 3.2 on Export and Tape Backup of the DELTA DMS User's Guide for information on the preparation of diskettes to transfer data to the CSCC. You are requested to purchase and use new, pre-formatted high density 3.5 inch diskettes for the export.

Tapes for backup files must be formatted and prepared for use by the DMS. Refer to section 6 on Backup Tape Preparation in the DELTA DMS User's Guide to format a tape for use.

7.2.3 Beginning to Use the DMS

The DMS was tested at the Coordinating Center prior to installation on each computer. Each time you turn on or reset your computer, several diagnostics are run to check for any problems or viruses. Following a successful start-up of the computer, a menu will be displayed to select the DMS or other software.

Refer to the DELTA DMS User's Guide for directions once you are in the DMS.

7.2.4 Reporting Problems

At any time during the start-up diagnostics or within the DMS that you receive an error message which informs you to call the Coordinating Center, *call us at once* to report the problem.

The contact at the CSCC is Nancy Anderson at 919-962-3052.

At the time of a problem call to the CSCC, a problem log will be completed and forwarded to the appropriate person for attention.

7.3 DATA CHECKING AND REPORTS

7.3.1 Display CXI

The CXI inventory form provides the number of each form type entered in the DMS. (See section 2.6 on Display CXI in the DELTA DMS User's Guide for more information.) The CXI inventory form can be viewed from the ID screen or from within any form screen. Either the inventory for a participant is shown if you have entered an ID, or an inventory of the entire database is shown if you have not entered an ID.

7.3.2 Search through Form Types

The Accept command has been renamed the Search command in the DMS to define more adequately the function of the command. You can use the Search command to scroll through every form type for a participant if you have entered an ID, or to scroll through every occurrence of a form type if you have not entered an ID.

7.3.3 Export Report

When you prepare the export file to send to the Coordinating Center, two reports are produced. The first report lists the key fields of each record exported, separately for each form type. The second report provides a summary of the number of records added, changed, and deleted for each form type.

A paper copy of the export report will be sent via FAX to DELTA Central Receiving at the CSCC on the day you prepare your export file on diskette. This will notify us that a diskette was prepared and mailed.

7.3.5 Basal Energy Expenditure Report

From the Reports option of the Main Menu, you can calculate the Harris-Benedict equations for basal energy expenditure (BEE) for the run-in phase and for randomization for the participants. Once the BEE is known for a participant, total calories is calculated for three physical activity levels of moderate activity, light activity, or sedentary to determine the calorie level to assign the participant for feeding.

7.4 DATA TRANSFER

7.4.1 Shipping Preparations and Schedule

Once every two weeks, with three exceptions for holidays, the field centers will run the DMS export facility of the DMS to send data to the Coordinating Center on diskette. Refer to the DELTA DMS User's Guide for instructions on how to prepare a transfer diskette for shipment. It is important that the diskettes be prepared on the dates indicated and that CSCC receive your data in a timely fashion. The schedule of diskette mailing dates for protocol 1 in 1993-94 is on the following page.

The CSCC expects to receive a diskette from each center within two working days of the shipping dates. All shipping dates fall on Friday, so diskettes are to arrive at the CSCC on Tuesday of the following week. If for some reason you have entered no data for a particular shipment interval, the CSCC needs to be informed so that we will not expect a diskette from you. *Even if you have no data to send and your diskette is empty, please run the DELTA DMS export procedure on the mailing date and send us a copy of your summary.* Each centers will send to the CSCC via FAX a paper copy of the export summary report on the scheduled Friday mailing date to inform the CSCC on whether or not to expect a diskette.

Special note: Lipid profile data will be keyed at the field centers following period 1 to arrive at the CSCC by the end of December 1993 for reporting to design the next protocol and then at the end of the study beginning in June 1994 to arrive at the CSCC by the end of July 1994. It is imperative that these data be entered promptly after the laboratory assays for timely statistical analyses.

7.4.2 Mailing Procedures

Use a sturdy foam-lined diskette mailer to send your diskette to the CSCC. Send the diskette on the scheduled Friday date for *two working day delivery* to the CSCC for arrival the following Tuesday. Again, use new, formatted 3.5 inch high density diskettes to export files to the CSCC.

Mailers should be addressed as follows:

DELTA Central Receiving
CSCC
Suite 203, NationsBank Plaza
137 E. Franklin Street
Chapel Hill, NC 27514

Send a paper copy of the export summary report via FAX to DELTA Central Receiving at the CSCC on the day you prepare your export file on diskette. This will notify us that a diskette was prepared and mailed.

1993-94 DELTA Protocol 1 Diskette Shipping Dates

1993 Oct 1
15
29

Nov 12

(*) Dec 3
17

(*) 1994 Jan 7
21

Feb 4
18

Mar 4
18

(*) Apr 8
22

May 6
20

Jun 3
17

Jul 1
15
29

(*) Allowance for holidays.

7.5 IN HOUSE DATA PROCESSING PROCEDURES

The Dietary Effects on Lipoprotein and Thrombogenic Activity (DELTA) study will receive most data from the field centers and central agencies on diskettes prepared from the EXPORT utility of the DELTA data management system (DMS). Diskettes will be sent on scheduled shipping dates once every two weeks. The files will contain all data records added or changed in the centers' database since the last data transfer.

7.5.1 Receipt and Processing of Data

1. File copies of the export summary reports sent via FAX to the Coordinating Center for notification of the shipment of a diskette. Record the center name, diskette filename, and date of receipt on the reports, and file these reports in the processing log. This report provides counts of all the data on the transfer by form type and transaction type (add, change, delete) and the run data of the export procedure.

2. On the day of receipt of the diskettes, record the information from the diskettes in the processing log. A file containing the export summary report received via FAX will accompany the diskette.

3. Within one working day of receipt of the diskettes, check the diskette for read errors. If errors are detected, contact the field center by telephone or eMAIL to resolve the problem. Maintain written reports of any correspondence with the centers.

4. If a diskette from a center has not been received after one day of the scheduled receipt date, contact the field center by telephone or eMAIL to inquire on the status of the data transfer. If the diskette has not been sent, ask the center personnel to send the diskette for next day arrival at the Coordinating Center. If the diskette has been delayed in shipping, ask the center personnel to check on the location of the diskette. If the diskette has been lost, have the center personnel contact Brian Stewart at the Coordinating Center for instructions on creating a replacement diskette.

5. An acknowledgement of receipt of the diskette at the Coordinating Center will be sent to the field center or sending agency within one working day of receipt.

6. When all errors are resolved the files are ready to upload to the study data base.

7.5.2 Data Transfer and Processing of Non-Standard Format Files

The central hemostasis laboratory will send non-standard format files on diskette to the Coordinating Center at the end of periods 1 and 3. These diskettes ***will not be*** processed by DTIMPORT but through special programming procedures by the systems programmers at the Coordinating Center to include these data on the study database.

1. Record the information in the processing log from the diskettes on the day of receipt of the diskette.

2. An acknowledgement of receipt of the diskette at the Coordinating Center will be sent to the field center or sending agency within one working day of receipt.

3. Forward the diskette to the systems programming staff at the Coordinating Center for processing.

7.5.3 Paper Forms Sent to the Coordinating Center

1. In the future, some data may be sent to the Coordinating Center on paper forms. Copies of the paper forms are retained at the field centers, and the original paper forms are sent in batches to the Coordinating Center. A shipping list will accompany the batch of forms identifying the contents in the batch.

2. Coordinating Center personnel will key data from the paper forms usually in the order of receipt. These data will be keyed within five working days of receipt or by the next scheduled arrival of paper forms.

3. A 100% verification (re-keying) will be performed on laboratory data. Verification is preferably done by a different person, or on different days if keyed by the same person. Verification will be done within three working days of the data entry. The person keying these data will work with the research staff to resolve any discrepancies.

4. After data are keyed and verified, data are prepared for processing by the EXPORT utility of the DMS. The data will be transferred to the study database following the procedures in section I.B. on processing data from the DMS.

5. DELTA paper forms contain confidential participant name and information and are to be stored in a secured filing cabinet or room. The file cabinet or room containing the paper forms should be a reasonable distance from the computer system and diskettes to minimize loss of data in case of a disaster.

   Only personnel who are authorized to enter data on the computerized data entry system should be given the Access Code and Password for the DMS to prevent unauthorized entry. If unauthorized persons have obtained the Access Code or Password, change these using the procedures described in the instructions for using the data entry system.

   Backup copies of all data files and diskettes should be maintained to avoid loss of data. The original and backup diskettes should be stored in a secured location, as far as conveniently feasible from the microcomputer, to reduce the risk of data loss.

   The Coordinating Center can restore data that has been received, but the centers must ensure that at least one of their copies of currently entered data (paper forms, files on diskettes or the computer system) will survive an occurrence of data loss such as computer theft, hard disk crash, automatic sprinklers, or coffee spills.

7.6 DELTA DATA MANAGEMENT SYSTEM INSTRUCTIONS FOR USE AND COMMANDS

DELTA Data Management System
User's Guide

Collaborative Studies Coordinating Center
University of North Carolina at Chapel Hill
Version 1 July 31, 1993

Table of Contents

7.6.1 Introduction

1.1 Function

The DELTA Data Management System (DMS) is a set of programs which manage data collected in the DELTA field centers. The DMS uses the Foxpro data base management system for screen display, data editing and storage.

The DMS provides several major functions:

Data Entry: Allows data to be keyed, edited and updated.

Data Transfer: Allows data to be sent to the DELTA Coordinating Center for inclusion in a consolidated database.

Reports: Provides counts of number of forms entered, lists of participants with missing forms, lists of unverified forms.

1.2 Starting the DMS

To run the DMS, type DELTA from the C:\ prompt. A menu will be displayed:

```
     Select one of the following

 F1   run DELTA Protocol 1 DMS
 F2   run Word for Windows
 F3   run pcAnywhere (CSCC calling)
 F4   run DaVinci email
 F5   send email to CSCC

 ESC  Exit to DOS
```

Press F1: Run DES

to start the data management system.

1.3 User interface standards

The DMS uses a combination of menus and function key commands to control its actions.

1.3.1 Keyboard and Mouse

The DMS uses the keyboard in a conventional way:

- The typewriter keys are used to type numbers, letters and symbols. All text should be entered in upper case.

- The cursor control arrow keys highlight menu bar options. Once the cursor is on a menu option, the Enter key either performs the action or brings up a submenu. The left and right arrow keys move within a field. The down arrow key and TAB move the cursor to the next field. The up arrow key and SHIFT+TAB move the cursor to the previous field. The Home and End keys move to the beginning and end of a field respectively. Page Up and Page Down move to the previous and next screens in a form. Ctrl+Page Up and Ctrl+Page Down move to the previous and next form.

- The ALT key moves the cursor to the menu bar.

- Most menu options have shortcut keys which are a combination of the ALT key and a letter, usually the highlighted letter. In this guide these are written as ALT+letter, for example ALT+e. To use the shortcut hold down the ALT key and simultaneously press the letter. Specific shortcut keys will be described when the menus are discussed.

- Most submenu options (the lists displayed after you choose a menu item) have shortcut keys which are usually a combination of the Ctrl key and a letter. To initiate an action with a shortcut key hold down the Ctrl key and simultaneously press the letter.

- F1 (function key 1) is the help key.

- F2 (function key 2) is the field duplication key.

- F3 (function key 3) is the list display key.

Menu items can be selected using the mouse. To select an item, move the pointer to the item and press the left mouse button once.

1.3.2 Menus

Most screens in the DMS have horizontal menu bars on the first line. These menus list the options available from the screen. To move the cursor to the menu, press the ALT key. Once the cursor is on the menu bar, there are two ways to select an option:

Use the left and right arrow keys to move the highlighted bar to the desired option and press ENTER.

Type the highlighted letter in the desired option. Once the bar is on the option, press ENTER to select it.

Some menu options have further choices which are displayed in a pull-down list when the option is selected. Use the up and down arrow keys to move the bar to the desired option or type the highlighted letter.

Shortcut keys have been defined for some menu and submenu options. To use a shortcut key, press the key combination while the cursor is in the data entry section of the screen. The cursor does not have to be on the menu. Shortcut keys for menu options are ALT and the highlighted letter of the options. Most shortcut keys for submenu options are a combination of the Ctrl key and a letter. The shortcut keys are displayed on the pulldown lists. Exceptions to these rules are the help key (F1), the field duplication key (F2), the list display key (F3) and the movement keys (Page Up, Page Down, Ctrl+Page Up, Ctrl+Page Down).

Under some conditions menu options are unavailable. For example if a user does not have delete privileges, the Delete option is not available. Unavailable options are not highlighted and cannot be selected.

1.3.3 Lists

Some fields, for example the form field on the ID screen, can be selected from master lists. When the cursor is on the form field, a list of all forms can be displayed using F3. Use the arrow, page up and page down keys to move the highlighted bar through the list. To select an item, place the highlighted bar on the item and press ENTER. The item under the bar will be put in the field.

1.3.4 Information and Warning Messages

Messages from the DMS are of two types. The first displays a message and tells you to press a key to continue:

```
Insert blank EXPORT disk in Drive B. - ESC to Exit
Press any key to continue....
```

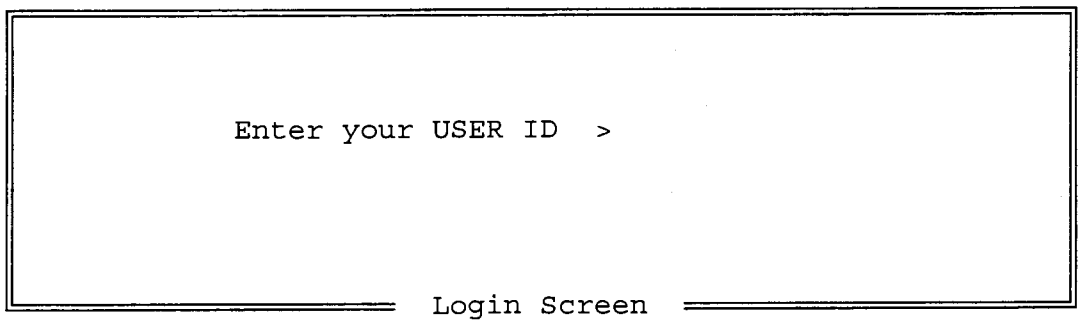
These usually appear when you must perform an action, like inserting a diskette. The message remains on screen until you press a key. The other type of message is used when no user action is required:

Login failure.....Please retry

It will disappear from the screen after a few seconds. However, you can make it disappear instantly by pressing a key.

1.4 Desktop, User IDs and Passwords

The password screen is the first DMS screen displayed:



At the top of the screen the menu bar lists two options:

Help presents a list from which you can select a DMS topic.

Desktop contains several features which are not vital to the DMS but which were included for your convenience. A pull-down menu is displayed when you choose Desktop:

```
Toggle Time     CTRL+T
Calendar/Diary  CTRL+D
Calculator      CTRL+C
Color Picker    CTRL+P
ASCII Chart     CTRL+A
Puzzle          CTRL+Z
```

The only selection which affects the DMS is the color picker. The color picker lets you define colors for the DMS. See Section 5 for a description of all the desktop utilities.

To do anything in the DMS other than Desktop utilities, you must enter a valid ID and password. These are assigned by using a password utility described later. IDs are three characters long and are displayed on the screen as you type. Passwords are at least three and at most eight characters long and are not shown on the screen. If you enter an invalid ID or password, an error message is displayed:

Login failure. Please retry.

The cursor is returned to the ID field so you can try again. After 3 failures the DMS quits.

If you want to leave the DMS from this screen, select **Quit** from the menu.

1.5 Timeout

At many field centers the DMS is started in the morning and not shut down until evening. This presents a security problem because a computer left unattended could be used to look at confidential data. To prevent this the DMS has a timeout feature. If no keys are pressed for 10 minutes, a message is displayed:

System timing out in 30 seconds.

Audible ticks count down from 30.

Press **ALT** to reset the clock and continue work. Note that it may take several rapid ALT key presses to effect a reset.

If you fail to press **ALT** before the 30 seconds expire, the system times out. The Password screen is displayed with a message that the system timed out. You must reenter your ID and password to resume using the DMS.

If a timeout occurs when a form is displayed, changes to the form are saved. A message is written to the Password screen indicating which form was active when the timeout occurred:

```
System timed out - No changes to active form - Last active
form was   Id:C00010     Form:EV1A  Timepoint:07/12/93
_____________________________________________________________

                  Press any key to continue....
```

If an ID and password are not entered within 30 seconds, a screen saver will put a random pattern on the screen. Press any key to stop the screen saver and return to the Password screen.

2 Data Entry

After you enter a valid ID and password, the DMS Main menu is displayed:

Data Entry **Utilities** **Reports** **Help** **Exit** **Quit**

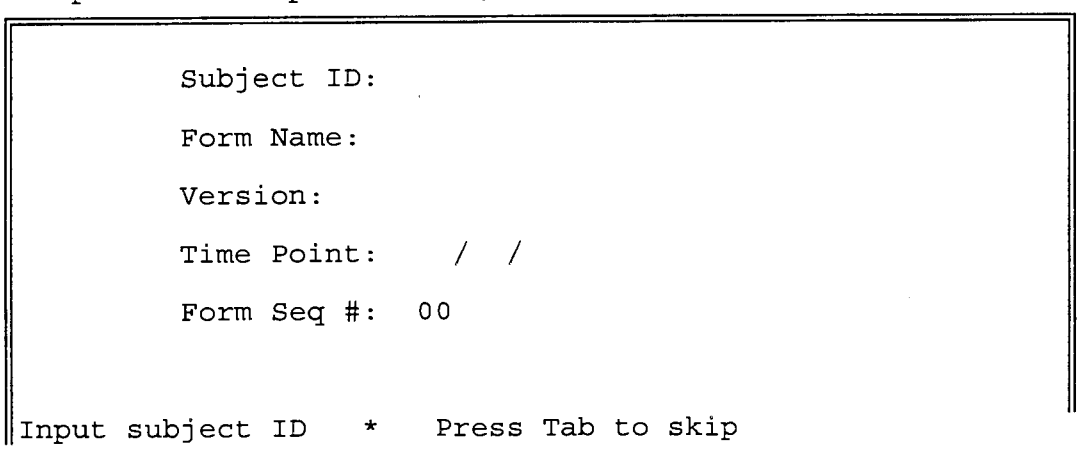
Select **Data Entry** to add and modify participant data. Select **Utilities** to run support programs such as the password program and the export and import programs. **Reports** runs report programs such as lists of missing or unverified forms. **Exit** returns to the Password screen. **Quit** leaves the DMS.

The highlighted letters of each option indicate which letter, in combination with the ALT key, comprises the shortcut. The shortcut key for Help is F1.

Press ALT+d to start the Data Entry System.

2.1 ID Screen

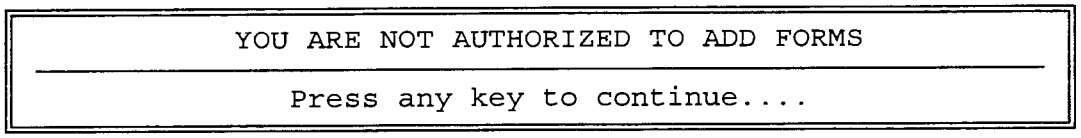
The ID screen is the first screen shown:



Entry mode, e.g. add, change or browse, is determined by which fields on the ID screen you enter, what is present in the database and some form specific rules.

If you enter all fields and the specified record is in the database, mode will be Change. In change mode you can modify fields on the form or delete the entire form. If you do not have modify privileges, mode will be Browse.

If a form with the specified keys is not in the database, mode is Add. An empty form is displayed for data entry. If you do not have Add privileges and are trying to add a form, you are told so:



The ID screen is redisplayed.

If you enter only some of the fields on the ID screen, the form with the keys which most closely match those fields entered is displayed for modification or browsing.

The following table summarizes the mode the data entry system assumes when certain fields are entered on the ID screen:

Fields Entered	Present in DB	Change OK	Mode
All fields	Yes	Yes	change
All fields	Yes	No	browse
All fields	No	Yes	add
All fields	No	No	not allowed

Fields Entered	Present in DB	Change OK	Mode
Some fields	Yes	Yes	change
Some fields	Yes	No	browse
Some fields	No	Yes	change w/ most closely matched form
Some fields	No	No	browse w/ most closely matched form

To summarize, to add a new form for a participant you must complete the ID, form and time point fields. (Sequence number defaults to 00.) To change records you can enter all or some of the fields on the ID screen.

As you enter the ID, edits check that it is a valid DELTA ID. If it is not, an error message is displayed and the cursor remains in the field. If it is valid, the cursor moves to the form field.

FORM can be entered in one of two ways. You can type a form abbreviation into the field, in which case the default or current version is automatically chosen and displayed in the version field. Or you can press the F3 key to display a list of all forms. Then TAB into the scrolling list of all forms, position the highlighted bar on the desired form and press ENTER. The form and version are plugged into their respective fields. Note that this second method is the only way to choose a version other than the default.

The cursor goes to the time point field. Enter the date the form was collected. Edits check that you have entered a valid date.

If sequence number is not 00, i.e. this is the second form of its kind entered on the same date, you must change the sequence number field first. When the screen is initially displayed, use SHIFT+TAB to go to the sequence field. Enter the appropriate sequence. Then fill in all other relevant fields.

You can leave the ID screen by one of two methods. If you have entered all fields, the requested form is automatically displayed when the last field is filled. However, if you want to leave the data entry session or return to the main menu, you must use the menu or a shortcut key to leave the ID screen.

The menu options are:

Accept: to display a form which most closely matches those fields entered.

Exit: to leave the ID screen and return to the Main Menu.

Quit: to leave the DMS.

2.2 Add / Browse Menu

When a form is displayed in Add or Browse mode, the top portion of the screen shows the key fields for the form. The cursor is on the first data field of the form. A menu bar fills the first line of the screen. The Add and Browse menus are identical with three exceptions: **Permanently Missing** applies only in Add mode; **Delete** and **Key Field Change** apply only in Browse mode. Some options on the Browse menu may not be highlighted. This means that they are not available. For example if you do not have delete privileges, **Delete** is not highlighted. If you do not have change privileges **Save** is not highlighted.

Add Menu:

Move **S**ave Cancel **P**roblem Help Display **P**erm.Miss

Browse Menu:

Move **S**ave Cancel **P**roblem Help Display **K**FChg. **D**elete

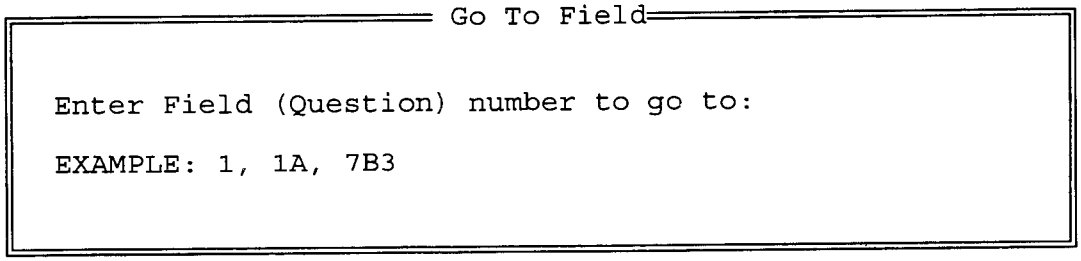
2.3 Field, Screen and Form Movement

In Add mode you will usually enter fields in sequence. However in both Browse and Add modes you can move through fields, screens and forms using the menus or shortcut keys. The Move option of the menu bar lists the available options:

Next Field	TAB
Prev Field	BACKTAB
Next Screen	PGDN
Prev Screen	PGUP
Next Form	CTRL+PGDN
Prev Form	CTRL+PGUP
Go to Field	CTRL+G
Switch Paths	CTRL+W

Most of the options are self-explanatory. **TAB** and **BACKTAB** move to the next and previous field respectively. **Page Up** and **Page Down** move to the next and previous screen of a form. **Ctrl+Page Up** and **Ctrl+Page Down** move to the next and previous forms in the current search order. If you go past the last form in the current search order, the first form will be shown again.

Go to Field allows you to move to a specific question on the form. Selecting this option brings up a menu in which you enter the question number to which you want to go:



If you enter an invalid number the message 'Field not found' will be displayed in the window. Enter another field or press ESC to return to the data screen.

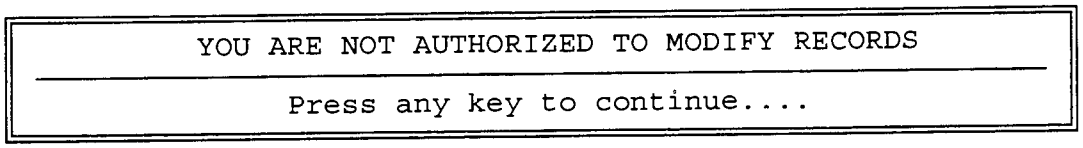
Go to Field allows you to go to skipped fields, permitting you to view screens which may have been skipped entirely. However you cannot enter values in these skipped fields. **Go to field** will not let you bypass a must enter field. If you enter a field after a must enter field which is blank, the cursor goes to the must enter field.

Switch paths allows you to control the order in which forms are presented when you select **Next Form** and **Previous Form**. The default order is by ID. With this path, the next form for the current ID is shown when you press **Next Form**. In Form order, the next ID for the current form type is shown when you press **Next Form**. Using form order, for example, you could view all LIP records which have been entered. Selecting **Switch Paths** toggles the path. Note that the path has no effect in Add mode since after saving or canceling a form, the ID screen is always displayed.

2.4 Edits

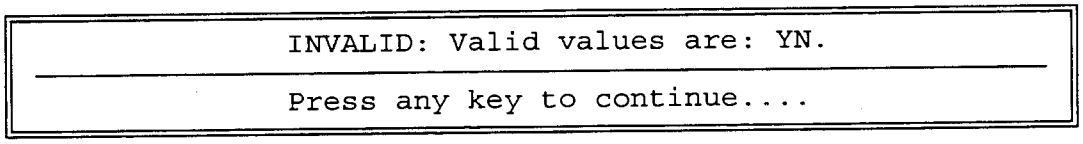
Each data field that you enter has an associated status byte vector which gives additional information about the field, for example, whether the field is empty, is missing, or contains an out of range value. The Problem selection on the Add and Browse menus gives you a way to provide this additional information.

As you enter data values into the form they are edited. If you do not have modify privileges, you will be alerted:



The old value will be replaced.

If a value fails an edit, for example if it is out of range or inconsistent with other values, an error window alerts you and gives the valid range:



Press any key to clear the error and return to the field. If you made a keying mistake, retype the value. However, if the value is correct you must confirm it. Use the Problem menu to do this:

Confirm value	CTRL+F
Questionable log	CTRL+Q
Note log	CTRL+N
Unresolvable field	CTRL+U
Reset field to blank	CTRL+T
Print form	CTRL+I

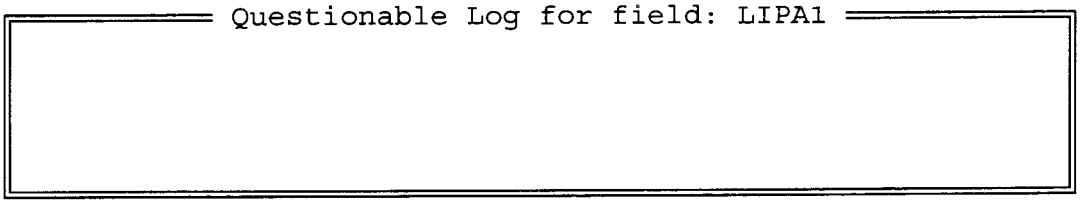
Choose **Confirm** to confirm that an out of range value is accurate by setting the first status byte to 'C'.

Use **Unresolvable** when a value cannot be collected or when the value you did collect is suspicious and should not be used in analysis. **Unresolvable** sets the first status byte to 'U' and, if the field is blank, fills the field with equal signs (==). Note that you can set a field to Unresolvable by keying the equal signs into the field rather than using the Problem menu.

Reset removes the value in the field and sets the first status byte back to 'E' (empty).

Questionable and **Note** allow you to comment on a field to explain a response or to enter a response that will not fit into the field. Choosing either option opens a window in which you can type comments.

Save Cancel Delete



If a note or questionable log has already been entered for the field, it is shown in the window. You can add to the end of the existing text. The window is empty if no log exists for the field.

There are four options on the menu bar. Choose **Delete** to delete the log displayed. To leave the log window without saving changes, select **Cancel**. Choose **Save** to save changes and return to the form. To print the log, choose **Print**.

Note and questionable logs function similarly but are used in different contexts. Use a questionable log when a value cannot be entered in a field, for example when the field is too short to hold the value. Use a note log to comment on a value, for example to explain an 'other' response.

Adding a note or questionable log set the third status byte to N for Note logs, Q for questionable logs or B for both.

When you enter the DES, the status bytes do not show on the screen. To display the status bytes, choose the Display option from the menu. The Display submenu is shown:

```
M  Matrix
F  Form
B  Disp SB CTRL+B
CXI        CTRL+C
```

Choose Disp SB. Or use the shortcut key 'Ctrl+b'. Once the status bytes are displayed choose the same option to turn off the display.

Only the first status byte of the vector associated with a field is displayed beside the field. When the cursor is on the field, the entire 3 byte vector is displayed in the upper right hand corner. The second byte of the vector is currently unused. The last byte indicates whether a note log ('N'), a questionable log ('Q') or both ('B') exist for the field.

2.5 Skips

Some fields are answered conditionally. That is, a certain response to one field can cause subsequent fields to be unnecessary or irrelevant. In the DES these fields are skipped. After a response is entered into the trigger field, the cursor automatically goes next relevant field. This field might be on the same screen or several screens ahead.

You cannot go into a skipped field using the field forward or field back keys. The only way to get to a skipped field is by using the Goto Field option on the Move submenu. Once on a skipped field, you cannot enter a value into the field.

The status bytes of skipped fields are changed to indicate the fields were skipped. The status byte values remain the same but are changed from upper to lower case.

2.6 Display CXI

The DES maintains an inventory of forms entered for each participant. This inventory form is called the CXI. It can be displayed from the ID screen or when a data entry form is on the screen.

From the ID screen, to show the CXI choose **CXI** from the menu (ALT+c). If you have entered an ID, the inventory for that participant is shown. If you have not entered an ID, an inventory of the entire database is shown.

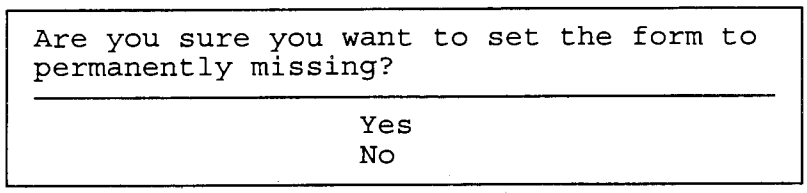
To show the CXI for the current participant from a data screen, choose **Display** from the menu. From the submenu select **CXI Display**. Or use the shortcut key 'Ctrl+c'.

The CXI display lists the numbers of each form type in the database.

2.7 Permanently Missing Forms

If you are unable to collect the information on a form for a participant, enter the form into the DES and set it to permanently missing. This tells the Coordinating Center staff that you will never be able to get the information so they will not ask you about it.

A form can be set to permanently missing only in Add mode. To set a form to permanently missing, choose **Perm. Miss.** from the Add menu. You are prompted to confirm the permanently missing:



If you have already entered data into some fields and then decide to set the form to permanently missing, the fields will be blanked. You are prompted to confirm again:

```
Form is not empty.  Do you still want to
set the form to permanently missing?
─────────────────────────────────────────
                    Yes
                    No
```

When a form is set to permanently missing, the first status bytes for all fields are set to 'M'. When browsing the database and a permanently missing form is shown, a message informs you that the form is permanently missing. You cannot add data to any field.

2.8 Delete

To delete a form, select **Delete** from the Browse Menu. There is no shortcut key for delete. You will be prompted to confirm the delete:

```
Confirm delete? Y/N
```

Type 'Y' to delete the record or 'N' to return to the screen. After you delete a record, the ID screen is redisplayed.

If you do not have delete privileges, the Delete option on the menu will not be highlighted. Delete privileges are granted via the system administrator utility.

2.9 Key Field Change

When a record is displayed for modification, most fields can be changed by simply entering a new value. However to change the key fields, the fields which identify the record, you must use KF Chg from the menu. With this option you can change the ID, the time point and the sequence number.

A screen similar to the ID screen will be shown:

Cancel Save

```
SubjID: C0000  Form:CLPA Time Point:12/12/92 Sequence # 00
   Search by ID                               Line # 00

        Subject ID:  C0000

        Form Name:   CLP

        Version:     A

        Time Point:  12/12/92

        Form Seq #:  00
```

Type in a new value for the field(s) you want to change.

If there is a form with the new keys in the database, the key field change is not accepted. When you are satisfied with the new values, go to the menu and select **Save**. To cancel the change, go to the menu and select **Cancel**.

The window will close and the form, with the new key fields, will be displayed. The Delete option is no longer highlighted. Deletes are not permitted after a key field change.

If you choose **Cancel** from the Browse menu, the key field changes will be lost.

If you do not have Change privileges **KFChg** on the Browse menu will not be highlighted.

2.10 Print Form

If you want a hard copy of a form, from the Browse or Add menu select **Problem Menu**. Choose **Print Form**. The following messages will be displayed:

```
Formatting printout - EV1A - Form as of 04/07/93 18:00:19
```

```
        Printing - EV1A - Form as of 04/07/93 18:00:19
```

The form with the data values you have entered will be printed.

2.11 Help

Help is an option on most of the primary menus in the DES. It can be selected from the menu or by pressing F1. **Help** from the Add or Browse menu

lists a submenu with three options: field, screen or general. Select the type you want. If you choose **General** a list of topics is presented. If you choose **Field** or **Screen** information specific to the current field or screen is presented. Once a screen is shown you can view related topics or select from a list of all help topics.

To return from help, press **ESC** or select **Exit** from the menu.

2.12 Save and Cancel

A form is automatically saved when you:

are in Add mode and enter the last field on a form; or

use ctrl+page up or ctrl+pg down to go to another form.

In the second case you are prompted:

```
Form was modified.  Do you want to save
the changes?
_____________________________________
                    Yes
                    No
```

in case you made changes inadvertently.

You can also save a form manually by selecting **Save** from the Add or Browse menu.

There are some situations in which you must manually save a form:

- When the response to a trigger field causes all remaining fields on a form to be skipped, you get the message

```
Can't move forward from current field
```

because there is no field for the cursor to move to.

- If you are in Add mode and must use an option from the problem menu on the last field of a form, you get the message

```
This is the last screen of the form
```

- If you are in change mode and want to return to the ID screen. Note that if you change the last field of the form in change mode, the form is not automatically saved.

In any of these cases, select **Save** to save the form and return to the ID screen.

Save is not available when you are in Browse only mode. See section 2.1 for a description of Browse Only mode.

If you have entered incorrect information and want to cancel all changes, choose **Cancel** from the menu. The ID screen will be displayed.

3 Utilities

The utilities are programs which are outside the DES but which affect how it runs or which act on the data entered using the DES. The utilities are run by selecting **Utilities** from the main menu. A submenu lists the available options:

Utilities

```
Sys Admin
Export data to CSCC  CTRL+X
```

3.1 System Administration

To use the DMS you must log in using an ID and password. You use the password utility to assign IDs, passwords and privileges to users. Generally this system administration task is assigned to a single person called the data coordinator.

To run the password utility. choose **Sys Admin** from the Utility submenu. Another submenu is shown giving four choices:

```
Add User
Delete User
Set Privileges
Change Passwords
```

To add new IDs and passwords, choose **Add User**:

```
                    ADDING USER

 Enter your USER ID > heb

 Enter new password >

 Enter new password again for verification >
```

Enter the user's login ID and password. Enter the password again for verification. Choose which privileges the user is allowed:

```
[x] Indicates a privilege is set ... Check quit to exit

[X] Report      [X] Browse      [X] Add      [X] Modify
[X] Delete      [X] Data Coor. Priv.

                [ ] Quit
```

Use the TAB key to move between options. Use the space bar add or remove an 'X' in the box. An 'X' in the box allows a privileges.

This screen is also used when you select 'Set Privileges' to change what a user is allowed to do in the DMS.

You can remove users from the system with **Delete User** and can change a user's password with **Change Passwords**.

There is a hierarchy of IDs which determines who is allowed to add and modify users and privileges. The Coordinating Center is on the first level, the data coordinator is on the second level and all other users are on the third level. A user can modify user IDs which are on a higher level. Thus the Coordinating Center can modify all users and the data coordinator can modify users on the third level. Users on the third level can only change their passwords.

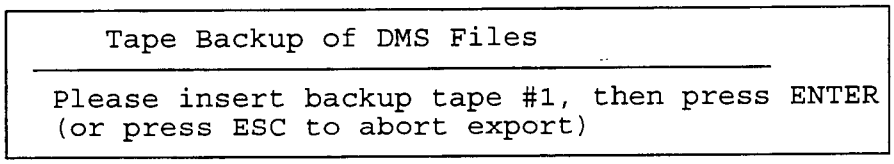
The DMS is shipped with a default Data Coordinator ID and password. You can add your own data coordinator ID by creating a new ID and assigning that ID data coordinator privileges.

3.2 Export and Tape Backup

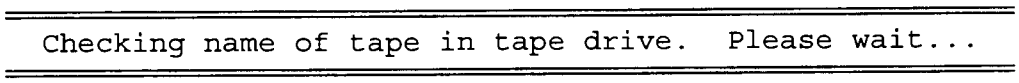
Once every two weeks data entered at the field centers will be sent to the Coordinating Center on diskette. The first step of the export is a backup to tape and the second is the creation of files to be sent to the CSCC. You will need one of the two backup tapes for the backup and a blank, formatted high density 3.5 inch diskette for the export.

To start the export, choose **Export Files to CSCC** from the **Utilities** menu.

The tape backup alternately uses one of the two backup tapes each week. As the first step in the export process you will be asked to insert the correct tape:



The system checks to make sure you have inserted the correct tape:



If the tape has not been formatted or prepared for use by the DMS, you will be notified and the backup will not continue. See section 6 for instructions on formatting and preparing tapes for use.

If you have inserted the wrong tape, you are notified and returned to the main menu. If the correct tape is in, the backup to tape proceeds. The files are first compressed and the compressed file is put on the tape.

After the tape backup, the export begins. You will be asked to insert a blank diskette in drive B for the transfer files. Use a formatted 3.5 inch high density diskette. Press a key when you have done so.

Two reports are produced. One lists the key fields of each record exported and is produced separately for each form. The second is a summary which gives the number of adds, changes and deletes for each form. You are asked whether you want to print the summary report. These reports are also copied onto the diskette.

The export files are compressed into a single file which is copied onto the diskette. The export file is named X000000n.zip, where X is the field center initial and n is the export file number. A message tells you the name of the file produced.

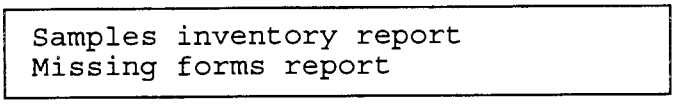
Please label the transfer diskette with this file name using the following format:

DELTA Export
Data Transfer
File Number X000000n
Date:

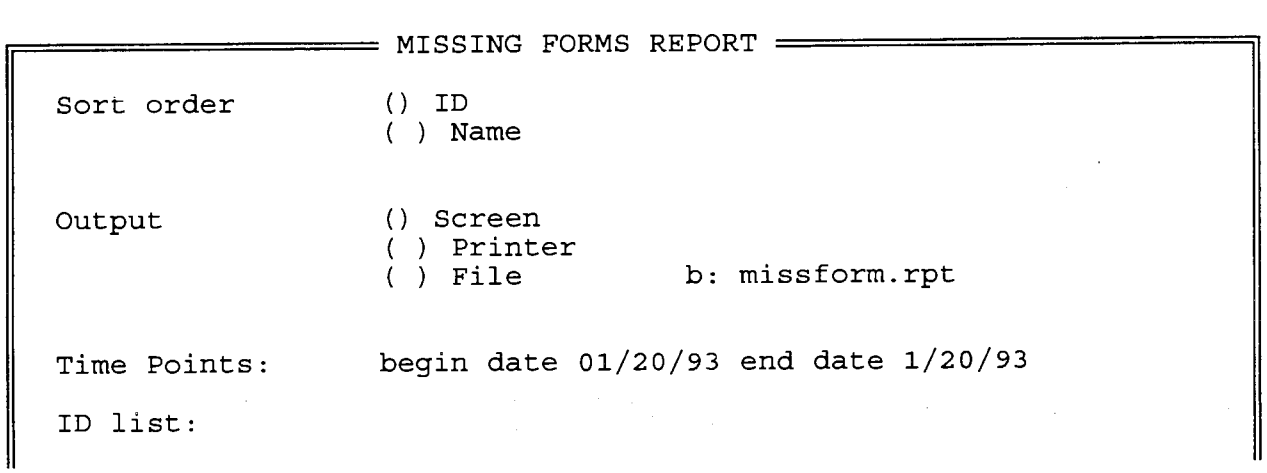
NOTE: If you get a fatal error during export, restart the export process using blank diskettes. You can later reformat any diskettes written to during the failed export process.

4 Reports

From the Main Menu choose **Reports** to display a list of reports available in the DMS:



Most reports require information such as subject ID, a date range on which to report or an output destination. The information screen is similar for all reports:



A single option can be selected from vertical lists (those preceded by parentheses ( )). To select an option, use the TAB or cursor control arrow keys to move to the desired option. Press the space bar or ENTER to select the option. This put a dot in the parentheses.

From horizontal lists (none are shown on above screen but would be preceded by brackets [ ]) multiple options can be chosen. Move to the desired options an use the space bar or ENTER key to select. This puts an X in the brackets.

Some options require additional input, like a file name or date range. The cursor will move to the required field automatically.

If you choose 'file' as an output destination, you must enter a file name. Notice that the file name is preceded by B:. This means the file will be written to diskette, so place a 3.5 inch diskette in drive B. If you choose printer, make sure the printer is turned on.

From this screen you can choose the Save or Cancel menu options displayed at the top of the screen to produce the report or cancel and return to the Main Menu.

4.1 Missing Forms Report

The missing forms report lists, for given participants, the forms which should be in the DES but are not. You can enter a single ID or a list of several IDs on which to report. You can also choose to send the report to the screen, the printer or a file on a diskette in drive B.

5 Desktop

On the Password Screen Menu is a Desktop option which contains some useful and fun programs which are not really part of the DMS but which might be useful. The Desktop submenu has these options:

Toggle Time	CTRL+T
Calendar/Diary	CTRL+D
Calculator	CTRL+C
Color Picker	CTRL+P
ASCII Chart	CTRL+A
Puzzle	CTRL+Z

Color picker is the only one which has an effect on the DMS. The others are completely separate from the DMS.

5.1 Color Picker

The DMS comes with preset colors. However you can modify the colors using the color picker. The process is complicated so if you want to do this, please contact the Coordinating Center for help.

5.2 Time, Calendar/Diary, Calculator, ASCII chart, Puzzle

Time toggles off and on the display of the current time in the upper right hand corner of the screen.

The calendar/diary presents a calendar of the current month and lets you record notes associated with a particular day:

Calendar/Diary
December 1992

Su	Mo	Tu	We	Th	Fr	Sa
		1	2	3	4	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30	31		

< M > < M > < Y > < Y

The calculator displays calculator which performs simple arithmetic. Use the mouse to 'press' the keys and compute sums, differences, etc.

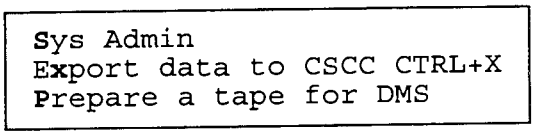
The ASCII chart shows the ASCII characters for keyboard keys.

The puzzle is a game in which you try to arrange the numbered blocks in ascending order by sliding a block into the open hole. Use the mouse to select and move a block.

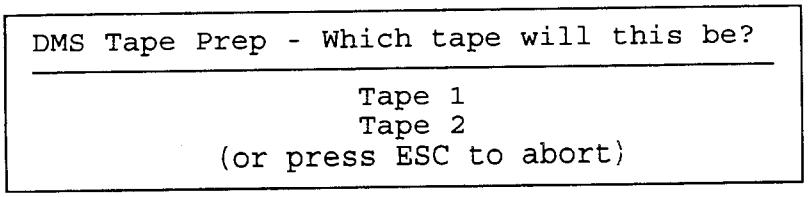
6 Backup Tape Preparation

Before tapes can be used to backup files they must be formatted and prepared for use by the DMS.

From the main menu select **Utilities** and from the **Utilities** pulldown menu select **Prepare a tape for DMS:**



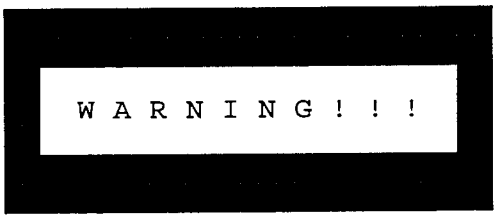
You will be asked which tape you are preparing. Remember that the DELTA DMS uses a cycle of two tapes, each one on alternate weeks.



Select which tape you are preparing. You will then be asked whether you want to format the tape. Generally only new tapes require formatting. However old tapes may be formatted to remove existing information.

```
Does this tape require formatting?
                    Yes
                    No
          (or press ESC to abort)
```

If you respond Yes, you will be warned:




```
TAPE WILL BE ERASED!  Continue?
                    Yes
                    No
          (or press ESC to abort)
```

You get the message:

```
Formatting Tape (This will take about 1 hour.)
   Tape format started at 3:23 pm, Monday, August 2, 1993
```

Formatting takes about one hour during which time you cannot do anything else on the pc. After the format is complete you will see the messages:

```
Preparing TAPE2 for DELTA DMS use. Please wait...
```

Old tapes have already been formatted and only require preparation for DMS use. Respond **No** when asked if the tape need formatting. You will get the messages:

```
Erasing tape (this will take about 2 minutes.)
     Tape erase started at 12:51 pm, Monday, August 2, 1993


Creating first volume on tape (this will take about 2 minutes.)
     Tape backup started at 12:52 pm, Monday, August 2, 1993
```

When these processes are finished, the main menu will be displayed. The tape is now ready for use. For instructions on using the tapes see section 3.2 Export.

7 pc-Anywhere

Sometimes the CSCC has to call the field center computers to diagnose or repair a problem. To allow the CSCC computer to control the field center computer we use a product called pc-Anywhere. To use pc-Anywhere, select F4: Run Remote Software from the DELTA menu.

The first screen gives a menu with several options. Select the first Begin Host Operation. Press ENTER.

The second screen gives another menu from which you select the first Wait For A Connection. Press ENTER.

The pc-Anywhere Connection status screen appears with the message

Initializing modem...

and then with the message

Waiting for connection...

The CSCC can then call your pc to fix problems. While we are working do not type anything unless instructed to do so. When we have finished we will hang up and Connection status screen will reappear with the same message

Waiting for connection...

Press ESC and the message

Operator abort
Press a key to continue

will appear. Press a key and the DELTA menu will be displayed.

To unload the program from memory, you must restart pc-Anywhere. Select F4: Run Remote Software again. From the first menu choose Begin Host Operation. Press Enter.

From the next menu, choose Exit and Remove from Memory. You will be returned to the DELTA menu.

8 E-mail

The DELTA sites are communicating with each other and with the Coordinating Center using an e-mail package called DaVinci mail. Please refer to the software user guide for assistance.

9 Word for Windows

To use Word for Windows, type DELTA from the C:\> prompt. From the DELTA menu select F2 Run Word.

Appendix A Keys Used in the Data Management System

Password screen

Desktop: alt-k

time	ctrl-t
diary	ctrl-d
calculator	ctrl-c
color picker	ctrl-p
ascii chart	ctrl-a
puzzle	ctrl-z

Quit: alt-q

Main menu

Data Entry: alt-d

Utilities: alt-u

Reports: alt-r

Quit: alt-q

Exit: alt-x

ID screen

CXI: alt-c

Accept: alt-a

Exit: alt-x

Quit: alt-q

Browse/Add

Move: alt-m

field forward	tab
field back	backtab
next screen	pgdn
previous screen	pgup
next form	ctrl+pgdn
previous form	ctrl+pgup
go to field	ctrl-g
switch paths	ctrl-w

Perm. Missing: alt-p

```
Problem:  alt-r
     confirm                ctrl-f
     questionable log       ctrl-q
     note log               ctrl-n
          Save:   alt-s
          Cancel: alt-n
          Delete: alt-d
     unresolvable           ctrl-u
     reset to empty         ctrl-t
     print form             ctrl-i

Display:alt-i
     toggle status byte display   ctrl-b
     CXI display                  ctrl-c

Cancel:  alt-n

Save:  alt-s

KF Change:  alt-k
     Save:  alt-s
     Cancel: alt-n

System-wide

     help:          F1
     Dup key:       F2
     Display list:  F3
```

CHAPTER 8

LABORATORY PROCEDURES

TABLE OF CONTENTS

CLINICAL LABORATORY MEASURES

I. OVERVIEW

A. Introduction

The Delta Study will examine the relationship between the levels of various substances in the blood as well as genes found in blood cells, and diets containing various combinations of fats, carbohydrates, etc. The blood samples will be extensively analyzed initially; storage samples will have further studies performed as new hypotheses and assays come forth. Because the blood samples collected are the foundation for all these tests, both present and future, the proper collection, processing, and storage of these samples is a crucial phase of the entire study. If the blood sample is not correctly drawn and processed, the laboratory results may not be precise or valid. Thus, the research assistant/research nurses who perform the blood drawing and sample processing must be well-trained, competent at drawing and processing the blood, and highly conscientious about the quality of their work.

The DELTA Study requires this considerable effort to standardize blood collection, processing, and storage, since it involves four U.S. field centers and at least five laboratories. If Columbia University and the Stanford/Taiwan group are successful in obtaining support for their collaboration, there will be a fifth field center in Taiwan and an additional laboratory at Stanford. This protocol is necessitated by the importance of all sample collection procedures being feasible and of high quality in order to meet all the investigators' needs. This protocol is derived, in part, from that developed by Russell Tracy, Ph.D., for the Cardiovascular Health Study, for which the University of Vermont serves as central laboratory.

B. Laboratory Organization

1. Description

The laboratories involved in the protocol will consist of those affiliated with the field centers, including Columbia University (CU), Louisianna State University (LSU), Pennsylvania State University/The Mary Imogene Bassett Research Institute (MIBH), Stanford University/Taiwan (SU), and the University of Minnesota (UM), as well as the central coagulation laboratory at the University of Vermont (UV) (Russell Tracy, Ph.D.). For the initial protocol, the following laboratories will perform the following analyses.

	LABORATORY					
ANALYTE	CU	LSU	MIBH	SU	UM	UV
Baseline Chemistry		x	x	x	x	x
Baseline TSH		x	x	x	x	x
Baseline Hematology		x	x	x	x	x

	LABORATORY					
ANALYTE	CU	LSU	MIBH	SU	UM	UV
Fasting Lipid Profile		x	x	x	x	x
Apoprotein A1				x		
Apoprotein B				x		
Lipoprotein (a)					x	
Baseline Apo E Genotype					x	
Fibrinogen						x
Factor VII						x
PAI-1						x

2. Rationale for Organization

a. Use of field laboratories for lipoprotein profiles

The rationale for the use of field center laboratories for the analyses of fasting lipid profiles deals with scientific, logistic, and financial issues. The alternative would be to use a central laboratory for this function.

First, the scientific rationale rests with the availability of a program to standardize measurements of all of these laboratories. The Centers for Disease Control Lipid Standardization Program has provided this service for NHLBI-supported laboratories for decades. Two of the laboratories (MIBH and UM) currently participate in this program. This provides the opportunity to decentralize the laboratories while developing and monitoring the program to minimize variance within precision goals.

Second, the effort of shipping these additional bloods to a centralized laboratory is alleviated. Local analyses must be performed for safety purposes (hematology, chemistry, TSH) so that local distribution of samples must be performed in any case. These will not require any standardization procedures.

Finally, most field center budgets have, as part of the personnel budget, support persons to do the sample collection, aliquoting, and storage. These personnel are needed regardless of where the analyses take place. The preservation of some laboratory charges at the field center allows the support for these essential personnel.

b. Pilot testing of field centers

Prior to the acceptance of a field center's ability to analyze its own lipid profiles, it must meet certification criteria of the CDC program. This will entail sending out blinded reference samples to all laboratories to be performed on four consecutive days (Appendix E). The results of these analyses will be compared between centers to assure that precision and accuracy are at acceptable levels; i.e., those which allow the study sample size to test major study hypotheses.

c. Selection of central laboratories for other analytes

The apolipoproteins (apo A-1, B, Lp (a)), apo-E genotypes, and coagulation factors (fibrinogen, factor VII, and PAI-1) currently have no standardization programs available to provide laboratory standards. Thus, the optimal way to control precision and accuracy is to use a central laboratory with recognized experience and methods. These laboratories were selected by the principal investigators from the field centers and coordinating centers, and program officers, after submission of competitive bids, based on precision, experience, and cost.

C. Rationale for Analytes Selected

1. Fasting lipoprotein profile

The levels of total cholesterol, HDL cholesterol, and triglycerides are analyzed in a standard fashion and constitute the analytes which are recognized as the serum lipids with greatest prediction of cardiovascular risk. LDL cholesterol is calculated using the Friedewald formula (LDL-C = TC-HDL-TG/5) because of a close correlation between LDL cholesterol measured directly using ulracentrifugation methods (in persons with triglyceride levels less than 400 mg per deciliter), and is much less costly than the laborious methods requiring ultracentrifugation. Because of the inclusion of triglycerides in the formula, all samples must be collected after an 8-hour fast.

2. Apolipoprotein A-1

Apolipoprotein A-1 is the major protein constituent of HDL and is suggested to be a better predictor of cardiovascular risk than HDL cholesterol, in some studies. It also serves as the co-factor in the lecithin cholesterol acyl transferase (LCAT) reaction. The observation that dietary components affect apo A-1 levels may provide insight into mechanisms of actions specific to dietary components.

3. Apolipoprotein B

Apolipoprotein B is the protein component of LDL and serves to recognize the LDL-receptor, allowing LDL particle uptake by cells. It has also been observed to be a better predictor of coronary disease than LDL-C in some studies. Low LDL cholesterol to apoprotein B ratio suggests the presence of small, dense LDL particles, which could be measured in subsequent studies.

4. Lipoprotein (a) (Lp(a))

Lipoprotein (a) is structurally similar to LDL, but with a covalently bound protein, apo(a), which has considerable homology with plasminogen. It is proposed to be a link between atherosclerosis and thrombosis. In fact, a number of cross-sectional and case-control studies have shown elevations in Lp(a) levels to be associated with clinical coronary, cerebrovascular, and peripheral vascular diseases. Relationships to dietary factors have been poorly studied, with a suggestion that trans fatty acids may elevate Lp(a) levels.

5. Apo-E genotypes

The hepatic E receptor binds chylomicron and VLDL remnants. People homozygous for the E-2 phenotype have reduced clearance of chylomicron and VLDL remnants. People homozygous for the E-2 phenotype have reduced clearance of chylomicron and VLDL remnants, resulting in some persons with dysbetalipoproteinemia (Type III). Three isoforms are recognized, E_2, E_3, and E_4. While patients with homozygous phenotypes (E_2/E_2) are recognized to have the above-described lipid abnormality, the E_4/E_4 homozygotes may also be associated with increased cholesterol levels, and it has been suggested to be associated with dietary responsiveness to fats and cholesterol. Thus, the phenotyping of each person may be important in describing the response to specific diets. Genotyping is proposed as a cheaper and more accurate method, which, while recently available, provides a clear improvement over the old isoelectric focusing methods. This needs to be done only once in each participating subject's white blood cell (buffy coat) DNA.

6. Fibrinogen

Concerning prospective cardiovascular risk, the major risk factor in the hemostasis area is fibrinogen. Northwick Park, Munster, ARIC, and the CHS studies have shown in large cohorts that fibrinogen is related to a variety of other risk factors, including smoking, race, white cell count, diabetes, and negatively, with HDL cholesterol. Northwick Park, Framingham, Munster, Goteborg, Leigh, Caerphilly, and Speedwell have <u>all</u> shown fibrinogen to be an independent, prospective risk factor for CVD. There is little evidence to link fibrinogen plasma levels to diet directly, but several arguments may be made for a potential indirect linkage. One of the key questions is: Is elevated fibrinogen the result of atherosclerosis and ongoing CVD, or a cause? While there is some genetic control of fibrinogen, it is primarily driven by environmental influences, including inflammation associated with acute or chronic conditions. Once elevated, it may cause increased atherosclerosis or thrombosis via one or several pathways: Increased fibrin formation per unit thrombin generation, increased platelet crosslinking, increased platelet viscosity, or decreased rate of fibrinolysis. All mechanisms have been observed in vitro, but the true in vivo story remains unclear. Since the general process of atherosclerosis is diet-related, then an intriguing hypothesis is that by altering diet, one may alter "inflammation" associated with atherogenesis which may, in turn, alter fibrinogen levels.

7. Factor VII

Factor VII has been related to cardiovascular disease cross-sectionally in a large number of studies, although not all (ARIC and CHS). There is only one prospective study

which lists Factor VII as a risk factor, Northwick Park. The second large study is Procam, and they have recently shown that there was a difference in baseline mean Factor VII values between those who developed CHD and those who did not, although this was not statistically significant. Factor VII has a certain genetic control, but this has not been addressed carefully. There is a well-recognized relationship between Factor VII activity and diet, and if major changes are made in the diet concerning fat level, there are clear changes in the Factor VII level in plasma, even in short-term experiments (two weeks or less). However, it remains unclear whether or not small dietary changes are a significant influence on Factor VII.

8. PAI-1

Plasminogen activator inhibitor-1 (PAI-1), a major plasma regulator of fibrinolysis, is associated with plasma lipids, especially triglycerides, and has been shown to be elevated in those with prevalent CVD. It is also elevated in those who go on to have a second myocardial infarction compared to those who do not. Other than that, there are no prospective data concerning PAI-1, but several studies are ongoing at the present time. PAI-1 is elevated in diabetics and some have proposed that this is due directly to plasma insulin. However, clinical studies have failed to reveal an acute response to insulin, so the insulin connection is currently in question. A second candidate is the plasma triglyceride level, since this is responsive to insulin to a certain extent, and might provide the link to insulin seen cross-sectionally. Since there is little known about the influence of diet on PAI-1, the studies proposed here may shed some light on this issue. PAI-1 levels appear to have a diurnal cycle, requiring the collection of samples between 7 and 10 a.m.

9. Baseline chemistry and TSH

Liver function tests (AST, ALT), renal profile (BUN, creatinine), glucose, and thyroid stimulating hormone (TSH) are performed at baseline to identify individuals with conditions which could modify dietary responsiveness to fats and cholesterol. Individuals who have these conditions should not participate in the study for both scientific and safety reasons.

10. Hematology

Hematocrit, hemoglobin, and white blood cell count should be monitored at baseline to assure that the subject will not suffer untoward effects from serial phlebotomy.

11. Other analytes considered

Additional analytes considered include glucose and insulin, platelet factor 4, and beta thromboglobulin, and a number of other coagulation parameters. For Study I, it was decided that the protocol for laboratory analyses would be rather simple. However, a large number of aliquots of serum plasma and buffy-coat cells will be carefully stored, to allow later analyses. Similarly, 24-hour urine collections will be of interest, but are put off to later studies, especially those which have fats which may affect prostaglandin metabolism.

II. BLOOD COLLECTION -- AN OVERVIEW

A. Overview

The DELTA Study requires collection of approximately 25.5 ml of blood from participants at baseline and approximately 36 ml of blood four times at the end of each diet phase. Additional samples may be required from premenopausal women. Since the study depends on the voluntary participation of subjects, both initially and in follow-up, every effort must be made to make the entire procedure as easy and painless as possible, both for the participants and for the field center personnel.

The following schematic describes the blood sampling for a single study subject (Figure 1).

The LDX Capillary Blood Analyzer by Cholestech Corporation (Walnut Creek, CA) will be used for subject screening. This instrument provides accurate and rapid (three minute) results for total and HDL cholesterol and triglyercides (if fasting) at reasonable cost. The Cholestech Corporation has agreed to donate one machine per field center and 500 cassettes at cost for this project. Publications which describe subject screenings should acknowledge use of this instrument in the Methods section and the contribution of Cholestech Corporation in the Acknowledgements. Technicians using this instrument should receive training by OSHA regulations, sample collection technique, and the proper use of this equipment.

Recruitment	Capillary Blood Total and HDL
Baseline	Baseline Package
Diet A, Week 5,6,7,8	Four endpoint packages
Diet B, Week 13,14,15,16	Four endpoint packages
Diet C, Week 21,22,23,24	Four endpoint packages

The baseline package contains serum for a chemistry profile and TSH (6 ml), EDTA plasma for hematology (3.5 ml), EDTA - plasma for buffy-coat (10 ml), and serum for a fasting lipoprotein profile (6 ml) for a total of 25.5 ml of whole blood.

The endpoint package contains a serum for lipid profile, apolipoproteins, and aliquots (9.5 ml), a tube for serum aliquots (9.5 ml), a citrate-plasma tube at room temperature (4.5 ml) for Factor VII, a second citrate-plasma tube at 4°C (4.5 ml) for fibrinogen and PAI-1, and an EDTA-plasma tube at 4°C (7.0 ml) for a total of 35 ml. For the endpoint package, any participants who are concerned about the volume of blood should be reassured that the total amount of blood is 1/15th of the volume given in a typical Red Cross blood collection (450 ml). Each endpoint package should be collected after an 8-hour fast with sample collection occurring between 7-10 a.m.

Serum was selected for analysis of lipoproteins inspite of the fact that many large studies have used plasma in the past. The reasons for this are severalfold. First, the EDTA used as anticoagulant extracts water from red cells, diluting the plasma somewhat. This problem translates into increased variability, since this process seems variable from person to person.

FIGURE 1: SCHEDULE OF CLINICAL LABORATORY MEASUREMENTS IN STUDY I

Measure	Type	Minimum Whole Blood Volume (ml)	Screening	Baseline	Week and Diet Endpoints A 5,6,7,8	B 5,6,7,8	C 5,6,7,8
Total Cholesterol	Capillary	0.05	x				
Chemistry Hematology	Serum EDTA Plasma	6.0 4.0		x x			
Lipid Profile	Serum	6.0		x			
Apo E Phenotype	EDTA Plasma	10.0		x			
Lipid Profile Apoproteins A-1,B Lp(a)	Serum	10.0			x,x,x,x	x,x,x,x	x,x,x,x
Fibrinogen PAI-1	Citrate Plasma	4.5			x,x,x,x	x,x,x,x	x,x,x,x
Factor VII	Citrate Plasma	4.5			x,x,x,x	x,x,x,x	x,x,x,x
Aliquots for Ancillary Studies	Serum	10.0			x,x,x,x	x,x,x,x	x,x x,x
Aliquots for Ancillary Studies	EDTA Plasma	7.0			x,x,x,x	x,x,x,x	x,x,x,x

FIGURE 2: SAMPLE PROCESSING FOR STUDY I

	BASELINE				ENDPOINT				
	1 Red	2 Red	3 Purple	4 Purple	1 Yellow/ Black	2 Blue	3 Blue	4 Purple	5 Yellow/ Black
VOLUME (ml)	6	6	4	10	10	4.5	4.5	7.0	10
IMMEDIATE TEMPERATURE	RT	RT	RT	RT	RT	RT	4°	4°	RT
Stage 1 Centrifugation (0-20 min)				Centrifuge RT 1500 g x 10 min	Centrifuge 4°C 3000 g x 10 min	Centrifuge RT 3000 g x 10 min	Centrifuge 4°C 3000 g x 10 min	Centrifuge 4°C 3000 g x 10 min	Centrifuge 4°C 3000 g x 10 min
Stage 2 Aliquot (10 min)				Buffy Coats (3x.5 ml ampules) Amber	7 Cryovial Red	4 Cryovial Green	4 Cryovial Blue	6 Cryovial Lavender	7 Cryovial Red
Stage 3 Freeze		x		x	x	x	x	x	x
Ship	Clin Chem	Field Lipid Lab	Clin Hem	Central Lipid Lab (1 Amber)	Field and Central Lipid Labs (4 Red Cryovial)	Central Coag (2 Green Cryovial)	Central Coag (2 Blue Cryovial)	None	None
Store -80°C				2 Amber Cryovial	3 Red Cryovial	2 Green Cryovial	2 Blue Cryovial	6 Lavender Cryovial	7 Red Cryovial

proto-5a;research

Second, fibrin fragments not pelletted with centrifugation can clog up analysis equipment, leading to errors and equipment failures.

An overall schema of sample processing is shown in figure 2.

B. Blood Collection Trays and Tubes

1. Blood Collection Tray

Blood collection trays are prepared in advance for the following day. Each tray is stocked with a full supply of blood-drawing equipment for three to four participants and holds individual blood collection racks for each participant in an ice bath. Several racks are prepared to hold various plastic tubes and vials for the final serum and plasma aliquots. The blood collection tube rack and aliquot tube rack are prelabeled with the appropriate code numbers for the participants drawn that day.

2. Blood Collection Rack

The collection tray itself is made of hard plastic which is unbreakable and can be easily cleaned. The tray has 10 individual compartments which will be filled with the following supplies:

21 g butterfly needles with luer adapter
alcohol swabs
Band-Aids
Gauze
Tourniquets (2)
Vacutainer holders
Needle/Sharps container
Smelling salts
Timer/Stop watch
Scissors
Adhesive tape
Styrofoam ice bath filled approximately
10 minutes before draw
Pencils/Pens
Latex gloves
Test tube racks (2)

A separate rack contains the necessary draw tubes, etc. for each participant. The tubes are arranged according to the priority of the draw. This rack will fit into the blood collection tray. The blood collection tubes are pre-labeled with identification numbers.

3. Description of blood collection tubes for baseline package

Tube 1 is a 6 ml siliconized orange-green stopper tube with serum separater. This contains no anticoagulants so that the blood clots to form serum. After drawing, the blood is allowed to clot at room temperature for 30-40 minutes. This tube is submitted to clinical chemistry for hepatic profile, renal profile, glucose, and TSH.

Tube 2 is a 3.5 ml lavender-stopper tube containing liquid 4.5 mM EDTA as the anticoagulant. After drawing, this tube is mixed by inverting and placed on a tube mixer. The tube will be submitted for hematology assay (hematocrit, hemoglobin, white blood cell count).

Tube 3 is a 10 ml lavender-stopper tube containing liquid 4.5 mM EDTA as the anticoagulant. After drawing, this tube is mixed by inverting on a tube mixer. It is centrifuged at R.T. for five minutes at 3,000 g. The buffy coat is removed and dispensed into three amber-coated vials for DNA analysis.

Tube 4 is a 6 ml siliconized orange-green stopper tube with serum separater. This contains no anticoagulants and is submitted to the field center lipid laboratory for baseline lipoprotein profile. This is centrifuged at 4°C at 3000 g x 10 min and 4-0.5 ml aliquots are frozen at -80°C before analysis.

4. Description of blood collection tubes for endpoint package

Tube 1 is a 10 ml siliconized yellow/black stopper tube. This tube contains thrombin so that the blood clots to form serum in 5 minutes. Aliquots are coded red. This serum is used for the lipid profile and apoproteins.

Tube 2 is also a 10 ml yellow/black stopper tube. It is processed the same as Tube 1 and is used for aliquots of serum for later studies.

Tube 3 is a 4.5 ml blue-topper tube containing 0.5 ml of 3.8% sodium citrate. It is crucial that this tube remain at room temperature. After centrifugation, plasma is aliquoted into green-coded aliquots. The plasma will be used for Factor VII coagulation assay.

Tube 4 is also a 4.5 ml blue-stopper tube identical to Tube 3. It is different in the way it is processed. Instead of room temperature, this tube must be placed on ice after filling. Plasma from this tube is aliquoted into blue-coated aliquots and will be used for fibrinogen assay and PAI-1 assay.

Tube 5 is a 7.0 ml purple stopper tube. This tube requires mixing after filling and placement on ice. After centrifugation, plasma is aliquotted into yellow-coded cryovials (0.5 ml). The plasma will be used for ancillary and supplemental studies.

Each draw tube is assigned a color-coated aliquot system. There is one type of aliquot used: 0.5 ml cryovials. The cryovials have colored caps.

5. Priority of tubes

A total of 25.5 ml of blood will be drawn from each participant for each baseline package. The tubes are drawn in the following order: Serum 6 ml - orange/red top, EDTA plasma 3.5 ml - purple top, EDTA plasma 10.0 ml - purple top, serum 6 ml - orange/red top. All four tubes are required, as they assess inclusion/ exclusion criteria.

The tubes are numbered 1-4 and arranged on the rack in order of draw. Samples from the four tubes are used in approximately eight different biochemical and hematological assays. The buffy-coated aliquots (three) obtained from baseline are amber/brown cryovials, approximately 0.5 ml in each cryovial.

A total of approximately 35 ml will be drawn from each participant in five tubes at each endpoint package. The tubes are drawn in the following order: Serum 10 ml - yellow/black top, serum 10 ml - yellow/black top, citrate plasma 4.5 ml - room temperature blue top, citrate plasma 4.5 ml - 4°C - blue top, and EDTA plasma 7.0 ml - 4°C. Tubes #1, 3, and 4 are of absolute priority and should be collected at each endpoint package, at the minimum.

The tubes are numbered 1-5 and arranged on the rack in order of draw (see diagram in this section). Samples from the five tubes are used in approximately 13 different biochemical and hematological assays.

6. Aliquot tube rack: Labeling and set up

Each participant will have two aliquot racks set up to correspond with the blood collection tube rack. Rack set up is completed the previous day. All tubes and vials are labeled and arranged appropriately (see diagram in this section). Aliquot assignments:

Polypropylene

Tube	Cryovials	Color Code
serum 10 ml	7 (0.5 ml)	red
serum 10 ml	7 (0.5 ml)	red
citrate 4.5 ml	4 (0.5 ml)	green
citrate 4.5 ml	4 (0.5 ml)	blue
EDTA plasma 7.0 ml	6 (0.5 ml)	lavender
TOTALS	28	

Each field center will be provided with a PC-based computer program to allow the inventory of aliquots stored at each field center to be tracked. Each field center will be responsible for the accurate maintenance of the inventory.

7. Forms

a. Purpose:

The purpose of the blood collection form is to facilitate the collection of plasma and serum samples from participants. This collection must be done in a rapid and efficient manner, with maximum protection for the participant. In addition, the process must facilitate the monitoring of phlebotomy and other quality assurance parameters as well.

Note: All forms are to be completed in ink.

b. Description:

There are two parts of the Phlebotomy Processing Form associated with blood drawing. The top half is the participant/phlebotomy question about fasting status. If the person has eaten less than eight hours earlier, he/she should be rescheduled on an alternate day, within three days of the initially scheduled date.

The bottom half deals with phlebotomy and processing of samples.

These forms have the following purposes: 1) Assure the most efficient and safest possible venipuncture for participants. 2) Allow the monitoring of the quality of the above procedures. 3) Allow more efficient processing of the samples at central laboratories. 4) Provide information critical to the interpretation of assay results.

c. Preparation for Specimen Collection:

Preparation for specimen collection is done in the following manner:

Early morning, prior to arrival of any participants:

1) Check to make sure that blood collection tray is properly equipped. Every item on the checklist must be ready before proceeding.

2) Check that each vacutainer tube is properly labeled with the appropriate participant number and number 1-4 (baseline) or 1-5 (endpoint) in order of draw. A sheet of numbered labels will be provided for each participant.

3) Check that the sample processing station is properly equipped. Every item on the checklist must be ready and in its proper position.

4) Check that each sample aliquot tube is labeled with its appropriate participant identification number and in its proper rack.

5) Check that the participant Phlebotomy Processing Forms are labeled and included with the blood collection tray.

6) Perform quality control check on refrigerator temperature (refrigerator temperature log).

7) Perform quality control check on freezer temperature (freezer temperature log).

8) Make sure the phlebotomy area is tidy and stocked with extra smelling salts, basin, disposable wash clothes, and that the blood mixer is functional.

Approximately 10 minutes before scheduled participant arrival:

1) Fill styrofoam bath 3/4 full with crushed ice.

At participant arrival:

1) Check that the ID number on the tubes matches the participant ID.

d. Venipuncture

1. Precautions for handling blood specimens:

In accordance with OSHA regulations on blood borne pathogens (see appendix for the complete OSHA regulations), the following laboratory safety protocol for field-center laboratories is recommended: Use of non-permeable lab coats, Latex gloves, and face shields when handling any blood in any situation where splashes, spray, splatter, or droplets of blood may be generated and eye, nose, mouth contamination can be reasonably anticipated. Follow universal precautions when handling any blood products. Contaminated needles and sharps shall be immediately placed in a puncture-resistant, leak-proof container. Never re-cap or break needles. Hepatitis B vaccine should be offered to all technicians handling blood.

2. Phlebotomy area:

The blood drawing area should take place in an isolated room or participants should be separated by room dividers. The room should be equipped with all the necessary blood drawing supplies. A separate counter or work cart should be equipped with all the materials and vials that are used for blood handling and processing. The centrifuge, refrigerator, and freezer should be near by.

3. Participant preparation:

Informed consent must be obtained before drawing blood. This procedure is followed to ensure that the subjects understand the purpose of blood drawing and the possible complications of venipuncture. A standard informed consent has been prepared for

this study. With regard to laboratory procedures, the consent statement informs study subjects that there is a small risk of bruising at the spot on the arm where the blood is taken and that about 2 tablespoons of blood are drawn. The consent statement also informs study subjects that they will be contacted if clinically important test results are abnormal.

4. Participant phlebotomy question:

Minimal fasting time required for testing is 8 hours (optimum is 12 hours), and all samples for endpoint package should be collected between 6-10 a.m. Record the time of last food and the time of blood drawing. If the participant is not fasting, the blood sample will not be drawn and the subject should be rescheduled for phlebotomy within the next three days. Similarly, if the collection time is outside the 6-10 a.m. period, the subject should be rescheduled for phlebotomy.

5. General:

Blood drawing is standardized for the sitting position. The venipuncture performed with the 21-gauge butterfly needle with 12 inches of plastic tubing between the venipuncture site and the blood collection tubes. The butterfly is a small, thin-walled needle which minimizes trauma to the skin and vein. The use of 12 inches of tube allows tubes to be changed without any movement of the needle in the vein. If the participant is concerned about the venipuncture, he/she may be reassured to know that such care is taken. The participant should be given enough time to feel comfortable both before and after the blood collection. In many cases, the most memorable part of the experience for the participant will be the contact with the person who draws the blood and their general attitude and competence. If the participant is nervous or excited, the technician briefly describes the procedure; e.g., "I am going to be drawing about 2 tablespoons of blood. This blood will be used for tests for lipids and cholesterol and blood clotting factors."

HANDLING PARTICIPANTS WHO ARE EXTREMELY APPREHENSIVE ABOUT HAVING BLOOD DRAWN.

Do not under any circumstances force a participant to have blood drawn. It may help to explain to the participant that the blood drawing is designed to be as nearly painless as possible. It is sometimes best to let the participant go on with another part of the examination. It may also be helpful to have the participant relax in the blood drawing chair just so the phlebotomist can check the veins in the participant's arms, without actually drawing blood.

6. Venipuncture procedure: Wear Latex gloves and lab coat

a) Arrange draw tubes in order of draw on the table top within easy reach. Assemble butterfly apparatus and vacutainer holders, gauze, and alcohol prep prior to tourniquet application.

b) Apply tourniquet to either arm.

c) Examine participant's arms for the best site for venipuncture. Release tourniquet.

d) Cleanse venipuncture site. Prepare area by wiping with alcohol swab in a circular motion from center to periphery. Allow area to dry.

e) Reapply tourniquet and start timer.

f) Grasp the participant's arms firmly using your thumb to draw the skin taut. This anchors the vein. The thumb should be one or two inches below the venipuncture site.

g) With the needle bevel upward, enter the vein with a smooth continuous motion.

h) Make sure the participant's arms are in a flat or dominant position while maintaining the tube below the site when the needle is in the vein. It might be helpful to have the participant make a fist with the opposite hand and place it under the elbow for support.

i) Grasp the sheath of the needle holder and push the tube forward until the butt end of the needle punctures the stopper, exposing the full length of the needle.

j) Note the blood flow into the first collection tube. If blood is flowing freely, the butterfly needle can be taped to the participant's arm for the duration of the study. If the blood flow is very slow, the needle may not be positioned correctly.

k) Remove the tourniquet at two minutes. Note the time on the PP form. Once the draw has started, do not change the position of the tube until it is withdrawn from the needle. If blood flow ceases after the tourniquet is removed, it may be reapplied for another two minutes, but this is noted on the Phlebotomy Processing Form.

l) Keep a constant, slight amount of pressure (in the direction of the needle) on the end of the tube (especially tubes #1 and #2). This prevents release of the shut-off valve and stopping of blood flow, do not bring pressure or reintroduce pressure after completion of the draw.

m) Fill each vacutainer tube as completely as possible; i.e., until the vacuum is exhausted and the blood flow ceases. If a vacutainer tube fills only partially, remove the vacutainer and attach another without removing the needle from the vein. As each tube is filled, mix by gently inverting before placing tube on the mixer. (See Section Blood Mixing During Venipuncture.)

n) When the blood flow ceases, remove the tube from the holder. Shut off valve recovers the point, stopping blood flow until the next tube is inserted (if necessary).

o) Average venipuncture time is 3-6 minutes, but any difficulties may increase this time to 10 or 15 minutes.

7. Removing the Needle:

To remove the needle, lightly place clean gauze over the venipuncture site. Remove the needle quickly and immediately apply pressure to the site with a gauze pad. Discard needle into puncture-proof sharps container. Have the participant hold the gauze pad firmly for 2 minutes to prevent a hematoma. Remove tube from the blood mixer and place on ice (#4,#5) and at room temperature (#1,2,3).

8. Bandaging the Arm:

a) Under Normal Conditions:

1) Set the gauze pad down over the site, continuing mild pressure.

2) Apply an adhesive or gauze bandage over the veni puncture site after making sure that blood flow is stopped.

3) Tell the patient to leave the bandage on for at least 15 minutes.

b) If the patient continues to bleed:

1) Apply pressure on the site with a gauze pad. Keep the arm elevated until the bleeding stops.

2) Wrap a gauze bandage tightly around the arm over the pad.

3) Tell the patient to leave the bandage on for at least 15 minutes.

9. Procedures for difficult draw and complications of blood drawing.

If a blood sample is not forthcoming, the following manipulations may be helpful.

a) If there is a sucking sound, turn the needle slightly or lift the holder in an effort to move the bevel away from the wall of the vein.

b) If no blood appears, move needle slightly in hope of entering vein. Do not probe. If not successful, release tourniquet and remove needle. A second attempt can be made on the other arm.

c) Loosen the tourniquet. It may have been applied too tightly, thereby stopping the blood flow. Apply the tourniquet loosely. If the tourniquet is the Velcro type, quickly release and press back together. Be sure, however, that the tourniquet remains on for no longer than 2 minutes at a time.

d) The phlebotomist should not attempt to venipuncture more than twice.

e) Reassure the participant that the inability to obtain a clean venipuncture is not any sign of a medical problem on his/her part.

f) If venipuncture is unsuccessful, the participant must be rescheduled at a later date with a different field center phlebotomist.

10. Syringe Technique for Venipuncture

Collection of blood sample using syringes may also be used if the phlebotomist, upon examination of the participant's veins, feels that sample will be difficult to obtain by vacutainer method. Blood collection using syringes should be used only if the phlebotomist anticipates a difficult draw (vein collapse, small veins, etc.).

11. Blood Mixing During Venipuncture:

Each tube should be treated as follows:

a) Serum (#1,2) - Invert once, place on rack at room temperature.

b) Citrate (#3) on mixer for 30 seconds then place on rack at room temperature.

c) Citrate (#4) on mixer for 30 seconds then place in ice bath.

d) EDTA (#5) on mixer for 30 seconds then place in ice.

12. Precautions:

a) When a participant feels faint or looks faint following the blood drawing:

1) Have the participant remain in a chair, if necessary, have him/her place head between knees.

2) Provide the person with a basin if he/she feels nausea.

3) Have the person stay reclined until the color returns and he/she feels better.

4) Place a cold wet cloth on the back of the person's neck.

5) If the person faints, use smelling salts to revive by crushing the ampule and waving it under the person's nose for a few seconds.

6) If the person continues to feel sick, contact the medical staff member who will advise you on further action.

13. Completing the Blood Draw Procedure:

a) Dispose of the needle and tubing:

Dispose of needle and tubing in the appropriate biohazard needle/sharps containers. (It may be necessary to use Hemostats to remove tubing from vacutainer holders.) Complete the first page of the Phlebotomy Processing Form. Clean up the venipuncture area (if necessary). Bring blood collection tray to the processing area with the filled vacutainer tubes.

Blood collection tray checklist per tray:

10 - 21 gauge butterfly with lure adaptors
10 - alcohol swabs
15 - Band-Aids
15 - gauze pads
5 - Vacutainer holders
2 - tourniquets
1 - smelling salts
1 - timer or stop watch
2 - pencil/pen
- Latex gloves
1 - hemostat
1 - adhesive tape
1 - scissors

Approximately 10 minutes before draw:

1 - styrofoam ice bath filled with ice.

Optional:

10 cc plastic syringes
20 cc plastic syringes
needles for syringes

Per participant:

1 - blood tube rack with four draw tubes (labeled and numbered)

1 - Phlebotomy Processing Form
Have available on phlebotomy cart:
basin
cold cloth
tube mixer
biohazard containers
needle/sharps container
paper towels

III. PROCESSING THE BLOOD SAMPLES

A. General

Processing should be done immediately following venipuncture. Personal protective equipment (non-permeable lab coats, double gloves with at least one Latex pair, spatter shield) must be worn. Flowchart is included at the end of this section to diagram this process (Figure 2).

B. Processing of Baseline Samples

The red stopper tube #1 (6 ml) with serum separator is placed on a rack at room temperature; the purple stopper tube #2 is mixed for 30 seconds and placed on a rack at room temperature. These tubes are submitted to the field center's chemistry and hematology laboratories. The buffy-coat aliquot is obtained from 10 ml EDTA tube #3 at baseline, after centrifugation at R.T. for 10 minutes at 3000 x g. After the plasma is removed, using an MLA pipette, carefully suction off the white cell layer. Try not to disturb the red cells. The buffy coat is aliquoted into three amber vials. The second red stopper serum tube (6 ml) with serum separator is also allowed to clot at room temperature for 30 minutes and then centrifuged x 3000 g for 10 minutes. The tube is then submitted to the field center's lipid laboratory for fasting lipid profile; it should be refrigerated if not delivered to the laboratory within three hours of phlebotomy. Alternatively, 4-0.5 ml aliquots can be frozen at -80°C prior to analyses at the lipid laboratory.

C. Immediate Processing of Endpoint Samples

Upon reaching the blood processing station, remove the blood tube drawing rack and ice bath containing tube from the blood collection tray. The rack should contain three tubes: #1, #2, #3. The ice bath should contain two tubes: #4, #5. All tubes should be processed within 20 minutes of phlebotomy. The corresponding aliquot racks (two per patient) should be ready. Rack #1 (with color codes red, blue, and lavender) should be placed in an ice bath. Rack #2 (with color code green) remains at room temperature.

D. Centrifugation

Tubes #1, #2, #4, and #5 are centrifuged at 4°C for 10-20 minutes at 3,000 g. The centrifuge may need to be precooled to 4°C. 4.5 ml and 7 ml balance tubes are required for tubes #4 and #5. Simultaneously, tube #3 is centrifuged at room temperature for 10-20 minutes at 3,000 g. A 4.5-ml balance tube is required. Once centrifuged, the maximum time allowed before aliquoting is 10 minutes. While these tubes are spinning, the blood collection tray can be restocked with tube rack and blood collection tubes, ice, and forms for the next participant. Recheck labels on the two aliquot racks. Perform any necessary clean-up.

E. Aliquots

Allow the centrifuge(s) to come to a complete stop. Remove tubes #1, #2, #4, and #5 from the 4°C centrifuge being careful not to shake the tubes. Tubes are put on ice. Remove tube #3 from the room temperature centrifuge. Place in tube rack at room temperature. Assess the plasma. Mark on the Phlebotomy Processing Form if lipemic (L), icteric (I), hemolyzed (H), clotted (C). Follow the outline on the Phlebotomy Processing Form for aliquoting the samples. Checkmark on the form if the aliquot is completed, and mark any different volumes. Be careful not to disturb the other white cell layers. If plasma is a lower than expected volume, use one less aliquot rather than disturb the red cells or buffy coat. Do not have any red cells in the aliquots. Use a new pipette tip for each tube type. Recap aliquots after each draw tube has been pipetted.

		ALIQUOT
Tube #5	Use 500 uL MLA Cryovials (6) - 0.5 mL plasma/cryovial	Rack 1 (on ice) Lavender code
Tube #4	Use 500 uL MLA Cryovials (4) - 0.5 mL plasma/cryovial	Rack 1 (on ice) Blue code
Tube #3	Use 500 uL MLA Cryovials (4) - 0.5 mL/cryovial	Rack 2 (room temp.) Green code
Tube #1	Use 500 uL MLA pipette Cryovials (7) - 0.5 mL serum/cryovial	Rack 1 (on ice) Red code
Tube #2	Use 500 uL MLA pipette Cryovials (7) - 0.5 mL serum/cryovial	Rack 1 (on ice) Red code

* Indicates minimum volumes specified are required.

Checkmark on the Phlebotomy Processing Form if the aliquot is complete, then mark any different volumes. Original blood collection tubes can be properly disposed of in biohazard waste bags.

F. Freezing

Upon completion of the processing steps, aliquots must be frozen within 10 minutes. Green aliquots (4 cryovials) at room temperature are added to the rack at 4°C. That rack is removed from the ice bath and placed upright in the freezer at -70°C for at least ½ hour (preferably until the end of the day). Make sure the aliquots are not wet when they are placed in the freezer. If the -80°C freezer is not immediately available, the aliquots can be placed on dry ice or snap frozen in liquid nitrogen/methanol mixture.

G. Completed Forms

The completed Phlebotomy Processing Form can be set aside in the daily work folder. These forms are copied. Originals are filed at each field center; copies are enclosed with each shipment of samples to the various laboratories.

H. End of the Day Procedures

Frozen aliquots and racks are packaged with prelabeled freezer box. One box contains the aliquots from a single endpoint series on each patient (28 aliquots per total). The boxes containing aliquots are stored at -80°C freezer by date. The copy of the Phlebotomy Processing Form is kept in a file to be included with the shipment of samples. Restock blood collection tray with samples. Label with next day's participants. Blood draw tubes, aliquots, forms. Arrange draw tubes and aliquots in their proper racks. Wipe down all work areas with 10% Clorox solution.

I. Summary of Processing Time Limitations

On blood drawn prior to processing:

1) Serum 9.5 ml - 40 minutes;
2) Serum 9.5 ml - 40 minutes;
3) Citrate 4.5 ml - room temperature - 30 minutes;
4) Citrate 4.5 ml - 4°C - 30 minutes;
5) EDTA 7.0 ml - 4°C - 30 minutes;

Once centrifuged, maximum time before aliquoting is 10 minutes; after aliquoting all samples, freeze within 10 minutes.

J. Sample Processing Checklist

Pipette: MLA 2,000
Pipette: MLA 1,000
Pipette: MLA 500
Pipette tips
Pasteur pipette with bulbs
Ice bath/ice
Latex gloves
Lab mat

Kimwipes
Biohazard waste bags
Clorox (10% in wash bottle)
Pen/pencils
Study participant aliquot racks (2) with prelabeled aliquots
Phlebotomy Processing Form (page 1 completed)
Freezer
Refrigerator
Centrifuges -- room temperature and 4°C
Balance tube
Thermometer
Zip-lock bag
Lab coats, gloves, face shields

IV. SHIPPING THE BLOOD SAMPLES

A. General

Blood samples are stored at the field centers at -80°C and shipped to the various laboratories according to the schedule in Figure 3.

B. Methods

The samples to be shipped are removed from boxes stored at -80°C and packaged in bags for each subject. Copies of the corresponding Phlebotomy Processing Forms should be sent for that shipment. A transmittal form is completed to include those samples that are actually included in the shipment. The proper mailing/shipping labels (provided by carrier) are filled out. The UV mailing address is: Laboratory for Clinical Biochemistry Research, Colchester Medical Research Facility, Room T-205, University of Vermont, 5580 South Park Drive, Colchester, VT 05446, phone number: (802) 656-8963 (Elaine Cornell). The MIBH mailing address is: Lipid Research Laboratory - 4th Floor, M.I. Bassett Research Institute, One Atwell Road, Cooperstown, NY 13326, phone number: (607) 547-3048 (Leslie Davidson). The styrofoam mailer is lined with absorbent material; i.e., paper towels. Approximately half the dry ice is placed on the bottom of the mailer. Check the ID number against the transmittal form. Carefully place the zip-lock bags, each containing the samples from a single subject, in the mailer. Remaining dry ice (6 pounds total) is placed on the sample. The top of the styrofoam is sealed up with tape. It is closed with the outer cardboard sheath. The Phlebotomy Processing Form is placed on top of the styrofoam before the outer sheath is closed by tape or strap. Fix shipping labels. Place the entire box in the refrigerator if pick-up is not immediate (samples should not be on dry ice for more than 24 hours). Do not ship samples for more than 30 participants per box. Note: The number of samples per box may vary depending on the size of the container used. We recommend using your judgment and 6 pounds of dry ice.

FIGURE 3: TIMELINE OF ARCHIVING AND SHIPPING

Samples	Archived Samples	Ship Date 9/25/93	12/1/93	6/1/94
Baseline (Aug.-Sept. 1993)	3 Amber	1 Amber MIBH		
Special Subjects* (Aug.-Sept. 1993)	14 red 6 green 6 blue 6 lavender	2 red MIBH 1 green UV 1 blue UV		
Endpoint				4 x 2 red MIBH
Diet Phase I x 4 weeks (10/25-11/20)	4 x 14 red 4 x 4 green 4 x 4 blue 4 x 6 lavender		4 x 2 red Field Lab 4 x 1 green UV 4 x 1 blue UV	4 x 2 red Field Lab 4 x 2 green UV 4 x 2 blue UV
Diet Phase II x 4 weeks (2/7/94-3/4/94)	4 x 14 red 4 x 4 green 4 x 4 blue 4 x 6 lavender			4 x 2 red MIBH 4 x 2 red Field Lab 4 x 2 green UV 4 x 2 blue UV
Diet Phase III x 4 weeks (5/2-5/28)	4 x 14 red 4 x 4 green 4 x 4 blue 4 x 6 lavender			4 x 2 red MIBH 4 x 2 red Field Lab 4 x 2 green UV 4 x 2 blue UV

*3 special subject/center (see Section VII E.)

C. Sample Shipping Checklist

Styrofoam mailing container with outer cardboard sleeve
Absorbent material
Zip-lock bag
Packaging tape
Dry ice (6 pounds per shipment)
Labels (provided by carrier)
Completed Phlebotomy Processing Form
Blood sample transmittal form

V. STORAGE OF ALIQUOTS

Blood samples sent to Field Lipid Laboratories, Central Lipid Laboratory (MIBH), and Central Coagulation Laboratory (UV) will be stored at -80°C until analyzed. This should be a brief period of time.

Numerous additional aliquots are to be stored at the Field Centers for later analyses, as yet not proposed. The rationales for using the Field Centers for storage:

1. This reduces the risk of loss of endpoint samples, since some samples would be retained by the Field Centers,

2. The labotories analyzing these samples are as yet unknown, and they could be shipped directly at the time needed.

Each Field Center should identify a -80°C freezer which is protected from thawing through a reliable alarm system or back-up power system. The loss of aliquots through thawing may result in exclusion of that center's samples from important analyses.

A software program will be developed for the purpose of inventory control. The maintenance of this inventory is the responsibility of the field centers. All samples collected, stored, and shipped shall be accounted for on this program. Training on the use of the program will be included in the training sessions.

VI. SAMPLE RECEIPT AND ANALYSES

A. Sample Receipt

Samples received at MIBH and UV will be preceded by a FAX of the transmittal form. Upon receipt of the shipment, the attached blood sample transmittal form is checked against the fax, to assure that no confusion has occurred and to allow for telephone verification if there are any lingering doubts. The samples are removed from the package and immediately frozen at -80°C for later analyses. Any problems in the shipment in terms of thawing, breakage, etc. should be noted.

Samples are thawed prior to analyses per protocol, either at room temperature or in a warming bath.

B. Lipid Profiles

1. Columbia University

All determinations of cholesterol and triglycerides in whole serum and in HDL will be carried out in the Core Lipid Laboratory of the Atherosclerosis Score at Columbia. Dr. Ginsberg is the Director of this laboratory, which is a participant in the Standardization Programs of the Centers for Disease Control. Cholesterol and triglyceride levels will be determined by enzymatic methods using the Hitachi 705 automated spectrophotometer. The interassay coefficients of variation for these two measurements are less than 3% at present. Whole HDL cholesterol will be measured at the precipitation of plasma apo-B-containing lipoproteins at 10 gm per liter Dextran sulfate and 0.5 M magnesium Mg Cl2 (0.91 mg per ml and 0.045 M final concentrations, respectively).

2. Louisiana State University

All routine lipid analyses (total cholesterol, triglycerides) will be performed on the Beckman Synchron CX5 automated chemistry analyzer. HDL cholesterol is performed in the Beckman TH5 after precipitation of the non-HDL fractions by Dextran sulfate (50,000 MW) (DMA, Dallas, TX) following the protocol of Warnick, et al. Assay controls by DMA are used to verify accuracy. LDL cholesterol is calculated using the Friedewald formula.

For automated analyses, daily quality control is performed prior to all analyses which will be run on that day. Acceptable results must be obtained on quality control before any results are allowed to be reported. The results are logged into a computer bases' quality control monitoring package (Lyphline, BioRad Laboratories). Results for the intralaboratory comparisons by BioRad for lipid analyses have been very good. For cholesterol, there is a cumulative CV of 1.5% for level 1 and 2.5% for level 2. Comparison with other Beckman CX5 users shows the SDI is -1.0 and -0.9 for levels 1 and 2, respectively (-2.0 and +2.0 acceptable). For triglyceride, there are cumulative CVs of 1.8% and 3.7% for levels 1 and 2. The SDIs are negative for 0.1 and 0.2 when results are compared to others CX5 users. For HDL, controls are assayed from DMA. They are consistently within the acceptable ranges for these values. CVs are 2.9% and 6.4% for levels 1 and 2.

3. The MI Bassett Research Institute

Lipid profiles consist of measurement of total cholesterol, triglycerides, HDL cholesterol after precipitation of apo-B-containing lipoproteins using 50,000 MW Dextran sulfate. LDL cholesterol is calculated today using the Friedewald formula. All analyses are done on a Roche MIRA random access automated analyzer. Cholesterol and HDL cholesterol are assayed by enzymatic method based on a cholesterol esterase and a cholesterol oxidase system using a peroxidase / 4 amino antipyrene detection system. This is manufactured as Roche reagent for cholesterol (Roche Diagnostic Systems). For HDL cholesterol detection, non-HDL lipoproteins are precipitated by treatment of 500 microliters of serum with 50 microliters of Dextran sulfate (Sigma Chemical). Triglycerides are assayed by Sigma Chemical triglyceride reagent based on hydrolysis by lipase and detection of glycerol by glycerol kinase, coupled with glycerol-1 -phosphate oxidation to produce peroxide, which is detected by peroxidase conversion of the aminoantipyrene to quinonemia.

Calculation of cholesterol is based on a serum-based calibrator certified for accuracy by cross-over with Abell-Kendall reference method. Calibration is done in triplicate once a month. Calibration of triglyceride is based on reaction with pure glycerol expressed as equivalent triolein (Sigma Calibrators). For total cholesterol, two levels of quality control material (from Dade) are included with each run. For HDL cholesterol, two levels of quality control material are precipitated and analyzed with each run. For triglyceride, two levels of quality control material are included in each run. A run is considered out of control, using Westgard rules and precision ranges based on MTP guidelines (3% for total choles terol, 5% for triglycerides, 3% for HDL cholesterol). If it runs out of control, the entire run is repeated.

4. University of Minnesota

Cholesterol is measured on a Roche Cobas FARA analyzer using Boehringer Mannheim enzymatic reagent. A frozen serum pool which has been measured on multiple occasions by the Abell-Kendeall method is used as calibreator. Accuracy of cholesterol measureemnts are also verified by the CDC and are in close agreement with their reference Abell-Kendeall method with fresh patient samples. Total serum triglyceride is measured on a Roche Cobas FARA analyzer using Boehringer Mannheim GB reagent and calibrator. This method gives a "true" triglyceride level which has been corrected for free glycerol concentrations. Triglyceride measurements are standardized by the Centers for Disease Control (CDC) Lipid Standardization Program and are in close agreement with the CDC reference triglyceride method. LDL cholesterol is estimated by the Friedewald formula. This equation assumes a ratio of five for plasma triglyceride to the VLDL cholesterol; while this ratio is correct for the typical American diet, it may change for other proposed research diets.

The HDL cholesterol is measured enzymatically after precipitation of VLDL and LDL with Dextran sulfate (molecular weight 50,000) and magnesium chloride. The method is the same as described by Warnick, et al. We have chosen the Dextran sulfate/magnesium precipitation method over the Heparin/Manganese procedure because Manganese interferes with enzymatic cholesterol methods and must, therefore, be removed with sodium bicarbonate. This adds additional steps and imprecision to the method. In addition, the Dextran/Magnesium method is less sensitive to variations in temperature and centrifugation.

C. <u>Apoprotein A-1 and BIOO (MIBH)</u>

The analytical method is rate nephelometry using antisera specific for Apo-A1 or Apo-B-100. The formation of antigen-antibody complexes are monitored with time; the initial reading of light scatter serves as a baseline for change. Instrument monitors the rate of change and rejects any sample where change in turbidity with time is outside an acceptable margin of error, thus assuring that the presence of particulate matter drifting through the measurement field or the occurrence of lipemia does not produce artificially modified results. Analysis is performed on a Beckman Array using Beckman reagents, calibrators, controls (Beckman Instrument Company, Fullerton, CA). Fresh or frozen serum may be used. Reagents are polyclonal antisera specific for either apolipoprotein A-1 or apolipoprotein B-100. Titers of the antisera are determined by the manufacturer and preparation is optimized for reaction in the volumes of buffer and sample used with the Array system. Each lot of antisera is accompanied by programming information which is entered into the instrument to fit the calibration curve and set the margin of error allowable for the rate of reaction. Calibrators are standardized to the internationally developed reference material designated by CDC-IUIS (Centers for Disease

Control and International Immunological Society). The instrument is calibrated every two weeks using this material. Control materials are included at the beginning, middle, and the end of all runs to assure reproducibility of results.

Quality control samples are included at the beginning and the end of each run and are analyzed in duplicate. If the average value is not within the +/-2 standard deviation range for the QC samples or if reproducibility in these samples is unacceptable (samples at the end of the run deviated by more than one standard deviation from those at the beginning), the entire run is repeated. The precision for Apoprotein A-1 at 117 mg per deciliter over the long-term (run to run) is 4.9% and within run is 0.7-1.8%. Apo-B 100 precision at 170 mg per deciliter over the long run (run to run) is 3.8% and within run is 0.3-0.9%. The samples that give an error signal, indicating that they fall outside an acceptable time course development of turbidity are diluted and reanalyzed to assure this reaction did not occur at antigen or antibody access.

D. Lipoprotein a (MIBH)

Analytical method is an ELISA (Enzyme Linked Immunosorbent Assay) using the Macra LPa kit manufactured by Terumo (Elkton, MD). Monoclonal antibody to Lp (a), immobilized on microtiter wells, serves as the capture antibody. Bound Lp (a) is detected using a monoclonal anti LP (a) antibody conjugated with horseradish peroxidase. The complex is detected and quantified by chromagen formation upon incubation of peroxide and o-phenylenediamine substrate. A 100 microliter aliquot of serum, stored at -80°C, is used for this assay. The antisera, calibrators, and controls are provided by Terumo (Elkton, MD) in their Macro LP (a) kit. ELISA plates are read on an automated ELISA plate reader, Dynatech model MR600 set to monitor at 492 nanomoles. A calibration curve, consisting of six ampules ranging from 0-80 mg/dL Lp (a) is run in each batch. Each calibrator is run in duplicate and averaged to generate a standard curve. Each sample is analyzed in duplicate.

Two quality control samples are included in each run. If these do not match the target value within +/-2 standard deviation, the run is repeated. The precision of LP(a) at 15 mg per deciliter on the long term (day-to-day) is 3.9% and within run is 1.4%. LP(a) of 36 mg per deciliter has a long-term precision (day-to-day) of 3.8% and within run of 1.8%. Samples of LP(a) concentrations above the highest calibrator or absorbing at over 2.0 absorbing units are diluted 1-to-1 with saline and repeated on another run.

E. Coagulation Factors

1. *Fibrinogen*

Fibrinogen will be measured by the clot-rate method of Clauss, using a semi-automated instrument, the ST4 from Stago. The assay will be standardized using the College of American Pathologists Standardized Reference Plasma, which the UV laboratory helped establish.

In addition to outside quality assurance programs, such as the Coagulation Program of the College of American Pathologists, quality control will be established with three control materials (normal, elevated, and low) and so-called Westgard Rule (the multi-rule Shewart QC System) will be applied. Longitudinal drift will be assessed using lyophilized

control plasma. In one recent large study in the UV laboratory, the fibrinogen assayed at an average monthly CV of approximately 3.09% and blind duplicate analyses included the technical error of 7.4% with a correlation calibration of 0.8727. The fibrinogen assay will be done each test plasma in duplicates at one dilution (1/10). If the fibrinogen concentration is too high, the sample is assayed again at 1/20, 1/40 dilution. Assay requires a minimal of 0.25 ml of citrated plasma.

2. *Factor VII Activity*

Factor VII activity will be assessed using one-stage clot-rate assay based upon the prothrombin time, using immunodeficient human plasma. The thromboplastin will be human placental thromboplastin, and the assay will be standardized using World Health Organization reference plasma. The semiautomated Coag-A-Mate X2 instrument from General Diagnostics will be used.

Quality control will be established with two control plasmas (both approximately normal) and so-called Westgard Rules (a multi-rule Shewart QC system) will be applied. Longitudinal drift will be assessed using lyophilized control plasma. In one recent large study in the UV laboratory, the Factor VII assay had a monthly average CV of approximately 5.31% and blind duplicate analyses indicated a technical error of 6.2% with a correlation of coefficient 0.9133. Factor VII assay will be done on each test plasma as singleton measurements on each of two dilutions, 1/20 and 1/40. If the Factor VII concentration is too high, the sample is assayed again at a higher dilution. The assay requires a minimum of 0.50 ml of citrated plasma. The plasma should be centrifuged as soon as possible following phlebotomy, using at least 30,000 g min of centrifugation. Ideally, the sample should not be cooled on ice following phlebotomy, nor should the centrifugation occur at 4°C, but rather at room temperature. Factor VII may be activated in the cold and this should be avoided whenever possible. Following preparation of plasma, the samples should be frozen as soon as possible. When thawed, the samples will be thawed quickly and assayed immediately to minimize the possibility of Factor VII activation.

3. *PAI-I*

PAI-I will be assayed in plasma using an ELISA method originally developed by Coller and colleagues. This method is sensitive to free and latent forms of PAI-I, but not complexed forms. The robotic system in our laboratory, Hewlett Packard Microassay System) automates virtually all aspects of this assay, including sample dilution, incubations, enzymatic color generation, absorbance reading, and data reduction. Quality control will be established with two control-plasma (both approximately normal), and so-called Westgard rules (a multi-rule Shewart QC System) will be applied. Longitudinal drift will be assessed using lyophilized control plasma. In routine use in the UV laboratory, this assay has a CV of approximately 9%. PAI-I ELISA will assay each sample at a fixed solution, in duplicate. If the sample results do not fall in an acceptable range based on a standard curve, then additional dilutions will be made and a sample rerun. The standard curve will be made from pooled plasma calibrated to agree with the laboratory of Dr. Desire Coller in Leuven, Belgium. There are no generally agreed upon standards at this time. The minimum sample of volume required is 0.25 ml of concentrate plasma, prepared as listed for fibrinogen.

F. Apoprotein E-Genotype (MIBH)

The analytical method is identification of the genotype based on the amplification of a key portion of the gene for apo-E using PCR (polymerase chain reaction) and identification of the gene (apo-E2, apo-E3, apo-E4) based on the pattern of DNA fragments produced by cleavage with the restriction enzyme (HhaI). This method is a modification of a published method (J.E. Hixson and D.T. Vernier, Restriction isotyping of human apolipoprotein-E by gene amplification with cleavage with HhaI, J Lipid Research 31:545-548, 1990). White blood cells from whole blood collected in EDTA serve as the source of DNA. One hundred microliter aliquot of whole blood is stored in a small centrifuge tube and 500 ul of a Tris-EDTA buffer is added to lyse the red blood cells. The sample is mixed by inversion and centrifuged in a table-top centrifuge to pellet the intact white blood cells. Supernatant is carefully removed, and the pellet is washed with Tris-EDTA buffer again. The super natant is discarded, and the resultant pellet is stored frozen. Reagents include Proteinase K (Signe Chemicals, St. Louis) for the isolation of DNA from leukocytes, TAQ polymerase (Promega) and the deoxynucleoside triphosphates (Boerhinger Mannheim) for amplification, the HhaI enzyme (Promega) for cleavage into fragments and standard pBR 322 DNA-Msp1 digest (New England Biolabs, Beverly, MA) to use as a standard marker on electrophonetic gels used to separate DNA fragments. We have prepared our own sense and antisense primers using the PCR-mate DNA synthesizer (Biorad).

G. Supplemental Analyses

If funds should become available, a number of additional analysis will be performed. These analyses will not require additional analyses, as sample collection and processing took these possible analyses into account.

1. LDL Subfractions (Louisiana State University - Pennington)

These analyses will require one EDTA-plasma cryovial (0.5 ml) from each endpoint package; 100 uL/assay is needed to be done in duplicate, for a total of 200 ml per endpoint on subjects from all four field centers.

LDL and HDL size distribution will be determined in frozen plasma samples obtained from each of the last three weeks of each dietary period from each subject. Size distribution will be determined by nondenaturing gradient gel electrophoresis as described by Musliner and Krauss (1) and Blanche et al. (2) with the exception that in-house 2-30% concave acrylamide gels will be used (3). The format of these gels is such that LDL and HDL size distribution can be determined from a single gel. All samples from a given individual will be analyzed at the same time on the same batch of gels and, to the extent possible, on the same gel. Two quality-control samples, obtained from single-use aliquots of frozen (-80°C) plasma, will be included on each gel. The quality-control plasma will be chosen to provide different LDL phenotypes and high and low HDL_2 (HDL_{2a}+HDL_{2b}) levels.

Gels will be stained with Sudan black B as described by McNamara et al. (4). The lipid distribution, as a function of gel migration (R_f) will be determined by densitometry at a resolution of 84 uM employing a BioRad GS-670 Imaging Densitometer. The R_f-based distribution will be converted to a particle size-based distribution employing the pardigm developed by Williams et al. (5) or by custom software available at this Field Center. From the

distribution of relative lipid-stain intensity versus particle diameter, the following parameter will be determined:

a. LDL phenotype (A, B, or Intermediate) based upon the peak diameter for LDL.

b. "LDL score" as defined by McNamara et al. (4) and based upon the weighted distribution of LDL among seven size classes as described by Krauss and Burke (1).

c. Relative LDL subpopulation distribution based upon Gaussian deconvolution (PeakFit Software) of the LDL pattern. Data will be expressed as percent of total LDL lipid staining intensity distributed among the seven LDL size classes.

d. Relative HDL subpopulation distribution based upon Gaussian deconvolution of the HDL pattern. Data will be expressed as percent of total HDL lipid staining intensity distributed among five HDL size classes as defined by Blanche et al. (2).

References

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Blanche, P.J., E.L. Gong, T.M. Forte, and A.V. Nichols. 1981. Characterization of human high-density lipoproteins by gradient gel electrophoresis. Biochem. Biophys. Acta 665:408-419.

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McNamara, J.R., H. Campos, J.M. Ordovas, J. Peterson, P.W.F. Wilson, and E.J. Schaefer. 1987. Effect of gender, age, and lipid status on low density lipoprotein subfraction distribution. Results from the Framingham Offspring Study. Arteriosclerosis 7:483-490.

Williams, P.T., R.M. Krauss, A.V. Nichols, K.M. Vranizan, and P.D.S. Wood. 1990. Identifying the predominant peak diameter of high-density and low-density lipoproteins by electrophoresis. J. Lipid Res. 31:1131-1139.

2. HDL Subfraction (Columbia University)

For assay of HDL-2/HDL-3, two or three EDTA-plasma cryovials (0.5 ml each) from each endpoint package will be needed. Each assay requires 500 uL and are done in duplicate per endpoint on subjects for all four field centers. HDL-2 cholesterol will be determined as the difference between whole HDL cholesterol and HDL-3 cholesterol after differential precipitation of HDL-2 from the dextran sulfate/Mg++ supernatant with 10 g/L dextran sulfate and 1.5 M $MgCl_2$ following the procedure of Gidez et al., J. Lipid Res. 23, 1206-1223 (1982).

3. LDL Resistance to Oxidation (University of Minnesota)

For assy of LDL resistance to oxidation, two serum cryovials (0.5 ml each) for each endpoint package will be required from subjects at all four field centers.

Measurement of LDL resistance to oxidation with hemin and H_2O_2. LDL (1.019-1.063 g/ml) will be isolated from 1 ml of serum by sequential ultracentrifugation. LDL will be oxidized with hemin and H_2O_2 in 96-well Immulon 1 microtiter plates (Dynatech, Chantilly, VA). The oxidation of LDL will be monitored by measuring the decreasing absorbance of hemin at 405 nm. The decrease in hemin absorbance parallels the increase in thiobarbituric acide reactive substances (TBARS) and conjugated dienes. TBARS will also be measured. The final assay concentrations for the micotiter assay will be 40 ug/ml LDL protein, 2.5 uM hemin, and 50 uM H_2O_2 in HEPES-NaCl (10-50mM) pH 7.4 buffer in a final assay volume of 0.15 ml. The assay will be started by the addition of H_2O_2. Each LDL sample will be assayed in quadruplicate. After the addition of hydrogen peroxide, the plate will be read at 43 second inter vals for four hours in a Vmax kinetic microtiter plate reader (Molecular Devices, Menlo Park, CA). The resistance of LDL to oxidation will be measured as the time required for LDL oxidation to reach maximum velocity, i.e., time to Vmax. The time to Vmax will be computed by computer software linked to the plate reader (Molecular Devices, Menlo Park, CA). The correlation (r) between lag time and time to Vmax in 56 samples was 0.992, however, time to Vmax was approximately 10 minutes longer than lag time. The analytic and analytic plus biologic coefficients of variation for the method were 5.9 and 9.6 percent, respectively.

4. Assays of Sex Hormones in Premenopausal Women

Three hormone assays will be performed on serum from premenopausal women: progesterone, estradiol, and luetinizing hormone. These will be used to assess the menstrual cycle and identify the particular phase (follicular, late follicular, and lutea) in order to assess any effect of the menstrual on the outcome parameters being measured. Two serum cryovials (0.5 ml each) will be required from premenopausal female subjects from two field centers (Penn State and LSU).

a. Progesterone

Progesterone will be measured by an RIA method using ^{125}I tracer (Count-a-Count from Diagnostic Products Corporation, Los Angeles, CA). This method is a solid phase immunoassay. The assay uses 10 ul of serum or plasma and the analyte is stable for six months at -80°C.

Reference ranges

Follicular	0.1 to 1.5 ng/mL
Luteal	2.5 to 28 ng/mL

b. Estradiol

Estradiol will be measured by an RIA method using ^{125}I tracer (Coat-a-Count from Diagnostic Products Corporation, Los Angeles, CA). This method is a solid phase

assay with virtually no cross reactivity with other steroid hormones. The assay uses 100 ul of serum or plasma and the analyte is stable for six months at -80°C.

Reference ranges

Early follicular	30-100 pg/ml
Late follicular	100-400 pg/ml
Luteal	50-150 pg/ml

c. Luetinizing Hormone (LH)

LH will be measured by an RI method using ^{125}I tracer (Coat-a-Count from Diagnostic Products Corporation, Los Angeles, CA). This method is a solid phase assay with virtually no cross reactivity with other steroid hormones. The assay uses 100 ul of serum or plasma and the analyte is stable for six months at -70°C. LH maintains a low and constant level in the blood during the follicular phase, rise rapidly at ovulation and fall to a constant level during the luteal phase. The rise of LH at ovulation is of about a three-day duration.

Reference ranges

Follicular	0-14 mIU/mL
Midcycle peak	20-70 mIU/mL
Luteal	0-16 mIU/mL

VII. QUALITY CONTROL PROCEDURES

A. Overview

To assure validity of the results in this study requires rigid adherence to collection, labeling, and shipping protocols. To ensure that these are well understood, a training session will be held to provide training and experience before the beginning of the project. Adherence to these protocols, including periodic reviews and retraining, are the responsibility of each field center.

In addition, other quality control measures will be taken to assess the integrity of the stored samples once they are removed from storage for analysis (sections VI.B and C.) in addition to individual laboratory quality control programs that are in place for each analyte (section VI.D.).

B. Sample Integrity

Aliquots of samples will be frozen upright at -80°C before being placed in boxes for long-term storage and shipping. Upon removal from freezers for shipping or analysis, the sample should be inspected to for evidence of thawing and leaking. If the liquid is no longer frozen (with top of frozen layer being horizontal in the vial) in the bottom of the vial or if there is evidence that the sample has leaked, based on less than the original volume remaining in the vial, the sample will be discarded and replaced by another sample that is acceptable.

Upon receipt at local or central laboratories, samples will be inspected upon unpacking to assure that they remained frozen in transit. Any sample showing evidence of being thawed will be noted. If transportation to the local labor atory occurs immediately upon removal from the -80°C freezer (within three hours) the sample needs to be kept frozen. Any sample that showed evidence of thawing but remained cold during transport may be analyzed for lipids and lipoproteins, but not for coagulation factors. Analysis should occur within three days of receipt of these samples. Any sample that leaked during transport will not be analyzed.

Any sample that has been identified as thawed will not be included in the data analysis if the analytical results are greater than 3SD (biologic plus analytical) from the average of the other three in that set of four.

Expected biologic plus analytical SD for triplicate analysis (Clin. Chem. 36,209, 1990)

	1 SD
Cholesterol	5%
Triglyceride	13%
HDL-chol	7%
Apo A-1	10%
Apo B-100	11%

Sample identity will also be verified based on packing lists and FAXed inventory lists. Any shipment that shows a discrepancy between the packing list and the actual contents may be sent back to the originating center to correct if the discrepancy cannot be resolved by a phone call.

C. Analytical Integrity

Samples will be coded such that all samples from a single subject can be identified and grouped into three sets, from each of the three diet phases. These will be analyzed for each analyte on a single run for the analyte. If any sample of a set from the end of a single diet trial is more than 3SD (biologic plus analytical) from the average of the others, it will be reanalyzed promptly to confirm the value. It is the responsibility of the laboratory performing each test to review results on a run to run basis to identify such outliers and schedule a repeat analysis.

D. Analytical Quality Control Procedures

All analytical runs will include appropriate quality control samples to assure the validity of the run. Quality control samples will be analyzed at specific intervals with each run as specified by the manual of operation for the laboratory performing the analysis. If the quality control materials do not meet the criteria set by the laboratory's manual of operation, the run must be rejected and repeated in total.

E. Longitudinal Control Procedures

To be able to assess comparability of analyses in earlier years and later years for this study, samples other than diet study samples, will be used as not to deplete the archival stores of study subject samples.

Three volunteers would be recruited from each center to provide a series of "endpoint" samples which would be treated and aliquoted as a modified endpoint set to provide six sets of vials for each of the core analytes. These would be drawn and processed in the same way as a regular endpoint series (Section III), except that two extra blue-top tubes will be drawn and processed, to assure adequate blue and green aliquots. A series would be sent for analysis with the samples at the end of the baseline/recruitment phase. The remainder would be held and anlyzed at the end of each successive diet trial for the core analytes - lipid profile, apolipoproteins A-1, B-100, and Lp(a) and for fibrinogen, PAI-1 and factor VII. These samples (12 in all) would reflect the comparability of results over the course of the entire project.

All field center laboratories will, in addition, participate in the Lipid Standardization Program of the Centers for Disease Control, which provides some control for longitudinal samples of lipoprotein profiles.

VIII. FORMS

A number of forms will be required for the tracking of sample collection and management of data (copies in Appendix F):

A. Baseline Lipid Profile Form

This form should be completed on all subjects from whom a baseline lipid profile is collected, and sent with the sample to the field center lipid laboratory.

B. Baseline Apoprotein E Genotyping Form

This form should be completed on all subjects from whom an Apo E Genotyping is collected at baseline, and a copy sent to the field center with the amber cryovial containing Buffy Coat.

IX. DATA RECORDING AND TRANSFER

A hard copy of all results will be retained by all laboratories for the duration of the study. Values from the field center lipid laboratories and the MIBH Lipid Laboratory will be submitted to each field center by FAX, regular mail, or electronically, using predesigned forms. The UV will send hard copy plus data entered on floppy disk.

X. ALERT VALUES

Results beyond these values should be flagged for review by the field center physician, who may elect to contact the patient's physician.

Assay	Call-Back Value
Creatinine	Above upper limit of normal
Hematocrit	Below lower limit of normal for age/sex
TSH	Above upper limit of normal
AST/ALT	Above 2x upper limit of normal
Total cholesterol (mg/dl)	greater than 200 mg/dl
Triglycerides	greater than 250 mg/dl (immediate contact if greater than 1,000 mg/dl)
Glucose (mg/dl)	less than 60, greater than 140

X. TRAINING PROCEDURES

A. Field Center Research Nurse/Technician Training and Certification

Field center technician training and certification is accomplished by a standardized training course at the Coordinating Center with performance monitoring at the individual field centers. This initial training includes a 1½-day seminar with both lecture and laboratory components. At the completion of the course, the technician will successfully complete a written and practical examination. The technician's performance is also evaluated at each field center by observation by the field center investigators and by periodic examination by supervisory personnel. Recertification of field technicians takes place prior to each blood-drawing year or when new technicians are enrolled.

B. Training Course by MIBH and UV Staff

The purpose of the training course is to provide standardized methodology for venipuncture and blood processing for the field centers. Training will also include collection of capillary blood specimens by finger-stick and use of the LDX. Standardization of procedures is important for the quality of blood samples from participants. The UV training session is preceded by a training video and consists of a three-hour lecture component followed by a four hour laboratory session, on day 1 of the course. The second day's activities are concerned with certification of field center technicians through a written and practical examination. Prior to the UV training courses, the field center technicians are required to review the field center manual of operations and view a training video of the procedures used for the sample collection.

C. Lecture Component Objectives

1. Overview and purpose of blood sample collection for UV

There will be a half-hour discussion of the importance of blood collection processing phases and the success of the DELTA Study. The role of the MIBH and UV laboratories in the study will be reviewed; some informational laboratory testing with participants is included.

2. Venipuncture techniques

This lecture presents information relating to the collection of blood samples: infection control, safety precautions including new OSHA regulations, management of the participant, special problems associated with finger-stick and venipuncture, handling of equipment, procedures for fingerstick and venipuncture, and completion of phlebotomy forms. Demonstration of finger-stick using LDX and venipuncture using the butterfly apparatus is seen.

3. Processing of blood samples

This lecture presents information on the proper procedures for processing venipuncture blood samples: purpose and proper management of each blood collection tube, aliquot rack setup, detailed instruction for the preparation of aliquots from each tube, centrifugation and temperature requirements for each tube, correct completion of blood processing form, and procedures for local storage.

4. Sample shipment to the central laboratories

This lecture presents information on shipment schedules and packaging of blood samples.

5. Quality assurance

a. Didactic session will be given which summarizes and explains all QC procedures which are to be used, blood collection, processing, and shipment.

D. Laboratory Component Objectives

1. Preparation for venipuncture

The field center research assistant/nurses prepare the blood collection trays and aliquot racks for blood drawing. The technician should be able to state the order of tube drawing, the purpose of each tube, the temperature of tube during and post phlebotomy, appropriate aliquot tubes for each collection tube, temperature, centrifugation, and the final number of aliquots to be forwarded.

2. Finger-stick and venipuncture on volunteer subjects

The technicians practice on one volunteer at a time following finger-stick and phlebotomy protocol. If the technician does not feel comfortable, he or she may observe for a longer period. At least one practice finger-stick and venipuncture will be done by each technician.

3. Sample processing

Following the venipuncture on each volunteer, the technician proceeds to process the samples as outlined in the manual. The technicians can practice sample

centrifugation and pipeting, gain familiarity with aliquot tube manipulation and color coding, and complete the processing form. This exercise will be supervised to assure proper procedural guidelines are understood by technicians.

4. Local storage and sample shipment

Once the processing is completed, the technicians place their aliquot racks in the freezer. Once samples have frozen, the aliquots are packaged in mailing containers and prepared for shipment.

E. Certification of Training

The second day of training is a half-day session. During the session UV and MIBH personnel evaluate the performance of the field center technicians. The technicians are given a written and practical examination. The written examination consists of multiple choice and true/false questions covering information presented in the field center manual. The practical examination requires performance of both phlebotomy on the volunteer and processing of the sample collection tubes. UV and MIBH supervisory personnel monitor the venipuncture and processing performance and evaluate the technician's performance using the standardized certification form. Both written and practical examinations must be successfully completed, as well as an on-site observation, in order for CHF certification to be granted to the technician. An example of the written examination and certification form are included in the appendix. Once the field center technician has been fully certified, including observation on-site, he/she is qualified to certify other technicians in the complete or partial process. The three steps for certification are:

1. Successful completion of written exam (prepared by UV).
2. Successful completion of practical exam (using certification form).
3. Observation by certified personnel of complete phlebotomy/processing procedures on the volunteer/participant.
   Completed written exams will be corrected and kept on file at the UV.

F. Monitoring Field Center Technicians

Supervisory personnel, such as the study coordinator, monitor the performance of the field center technician through observation of phlebotomy on participants at each field center. Visits to each field center during the study provide a quality assurance check on blood collection and processing of participant samples. Review of blood collection forms by MIBH/UV will also be done, providing additional monitoring of field center activities.

G. Maintaining Certification

In order to maintain certification, the technician must complete phlebotomy and processing in one-full-day's worth of participants every two week period, not necessarily on a single day.

DELTA
Field Center Laboratory
Training session

Quiz

University of Minnesota
July 26-27, 1993

True or False

__ 1. The DELTA project will use laboratory results both as entry criteria and as endpo nts in this trial.

__ 2. Any phlebotomist with 6 months of experience is qualified to draw blood samples for the endpoint samples.

__ 3. Subjects must be fasting at least 8 hours (preferably 12) for all blood samples.

__ 4. For endpoint samples, the tourniquet should be on for a maximum of 2 minutes.

__ 5. All blood collection tubes must be mixed well (30 sec) immediately upon collectic ı.

__ 6. If a sample is mislabeled at collection (wrong subject, wrong week or wrong orde of samples) there is no way for that error to be detected and corrected.

Choose the correct answer(s)

1. The phlebotomist should not attempt venipuncture more than
   a. once
   b. once in each arm; total twice
   c. twice in each arm; total four
   d. it takes to fill the first four tubes; the fifth tube is not as necessary since it is for archiving only.

2. Ideal time from blood collection to centrifugation for processing is
   a. 4 minutes
   b. 15-30 min
   c. 1 hour
   d. 3 hours

XI. The Standard

General Industry

Part 1910 of title 29 of the Code of Federal Regulations is amended as follows:

PART 1910—[AMENDED]

Subpart Z—[Amended]

1. The general authority citation for subpart Z of 29 CFR part 1910 continues to read as follows and a new citation for § 1910.1030 is added:

Authority: Secs. 6 and 8, Occupational Safety and Health Act, 29 U.S.C. 655, 657, Secretary of Labor's Orders Nos. 12-71 (36 FR 8754), 8-76 (41 FR 25059), or 9-83 (48 FR 35736), as applicable; and 29 CFR part 1911.

* * * * *

Section 1910.1030 also issued under 29 U.S.C. 653.

* * * * *

2. Section 1910.1030 is added to read as follows:

§ 1910.1030 Bloodborne Pathogens.

(a) *Scope and Application.* This section applies to all occupational exposure to blood or other potentially infectious materials as defined by paragraph (b) of this section.

(b) *Definitions.* For purposes of this section, the following shall apply:

Assistant Secretary means the Assistant Secretary of Labor for Occupational Safety and Health, or designated representative.

Blood means human blood, human blood components, and products made from human blood.

Bloodborne Pathogens means pathogenic microorganisms that are present in human blood and can cause disease in humans. These pathogens include, but are not limited to, hepatitis B virus (HBV) and human immunodeficiency virus (HIV).

Clinical Laboratory means a workplace where diagnostic or other screening procedures are performed on blood or other potentially infectious materials.

Contaminated means the presence or the reasonably anticipated presence of blood or other potentially infectious materials on an item or surface.

Contaminated Laundry means laundry which has been soiled with blood or other potentially infectious materials or may contain sharps.

Contaminated Sharps means any contaminated object that can penetrate the skin including, but not limited to, needles, scalpels, broken glass, broken capillary tubes, and exposed ends of dental wires.

Decontamination means the use of physical or chemical means to remove, inactivate, or destroy bloodborne pathogens on a surface or item to the point where they are no longer capable of transmitting infectious particles and the surface or item is rendered safe for handling, use, or disposal.

Director means the Director of the National Institute for Occupational Safety and Health, U.S. Department of Health and Human Services, or designated representative.

Engineering Controls means controls (e.g., sharps disposal containers, self-sheathing needles) that isolate or remove the bloodborne pathogens hazard from the workplace.

Exposure Incident means a specific eye, mouth, other mucous membrane, non-intact skin, or parenteral contact with blood or other potentially infectious materials that results from the performance of an employee's duties.

Handwashing Facilities means a facility providing an adequate supply of running potable water, soap and single use towels or hot air drying machines.

Licensed Healthcare Professional is a person whose legally permitted scope of practice allows him or her to independently perform the activities required by paragraph (f) Hepatitis B Vaccination and Post-exposure Evaluation and Follow-up.

HBV means hepatitis B virus.

HIV means human immunodeficiency virus.

Occupational Exposure means reasonably anticipated skin, eye, mucous membrane, or parenteral contact with blood or other potentially infectious materials that may result from the performance of an employee's duties.

Other Potentially Infectious Materials means

(1) The following human body fluids: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluid, saliva in dental procedures, any body fluid that is visibly contaminated with blood, and all body fluids in situations where it is difficult or impossible to differentiate between body fluids;

(2) Any unfixed tissue or organ (other than intact skin) from a human (living or dead); and

(3) HIV-containing cell or tissue cultures, organ cultures, and HIV- or HBV-containing culture medium or other solutions; and blood, organs, or other tissues from experimental animals infected with HIV or HBV.

Parenteral means piercing mucous membranes or the skin barrier through such events as needlesticks, human bites, cuts, and abrasions.

Personal Protective Equipme is specialized clothing or equipme: t worn by an employee for protection a ainst(hazard. General work clothes (e g., uniforms, pants, shirts or blouse) not intended to function as protecti n against a hazard are not consid ed to be personal protective equipme t.

Production Facility means a f cility engaged in industrial-scale, larg - volume or high concentration pr duction of HIV or HBV.

Regulated Waste means liqui or semi-liquid blood or other poten ially infectious materials; contaminat d items that would release blood or othe potentially infectious materials a liquid or semi-liquid state if com pressed; items that are caked with dried lood or other potentially infectious mate ials and are capable of releasing the e materials during handling; conta ninated sharps; and pathological and microbiological wastes containi g blood or other potentially infectious m terials.

Research Laboratory means a laboratory producing or using re earch-laboratory-scale amounts of HI or HBV. Research laboratories ma produce high concentrations of I IV or HBV but not in the volume foun in production facilities.

Source Individual means any individual, living or dead, whos blood or other potentially infectious m terials may be a source of occupational exposure to the employee. Exam les include, but are not limited to, h spital and clinic patients; clients in ins itutions for the developmentally disable trauma victims; clients of drug a d alcohol treatment facilities; resi ents of hospices and nursing homes; hu an remains; and individuals who d ate or sell blood or blood components.

Sterilize means the use of a p ysical or chemical procedure to destro all microbial life including highly re istant bacterial endospores.

Universal Precautions is an a proach to infection control. According t the concept of Universal Precaution all human blood and certain human body fluids are treated as if known to e infectious for HIV, HBV, and oth r bloodborne pathogens.

Work Practice Controls mean controls that reduce the likelihoo of exposure by altering the manner n which a task is performed (e.g., prohibiting recapping of needles y a two-handed technique).

(c) *Exposure control*—(1) *Expo ure Control Plan.* (i) Each employer avi an employee(s) with occupation exposure as defined by paragra h (b) of this section shall establish a wri en Exposure Control Plan designed o

eliminate or minimize employee exposure.

(ii) The Exposure Control Plan shall contain at least the following elements:

(A) The exposure determination required by paragraph(c)(2),

(B) The schedule and method of implementation for paragraphs (d) Methods of Compliance, (e) HIV and HBV Research Laboratories and Production Facilities, (f) Hepatitis B Vaccination and Post-Exposure Evaluation and Follow-up, (g) Communication of Hazards to Employees, and (h) Recordkeeping, of this standard, and

(C) The procedure for the evaluation of circumstances surrounding exposure incidents as required by paragraph (f)(3)(i) of this standard.

(iii) Each employer shall ensure that a copy of the Exposure Control Plan is accessible to employees in accordance with 29 CFR 1910.20(e).

(iv) The Exposure Control Plan shall be reviewed and updated at least annually and whenever necessary to reflect new or modified tasks and procedures which affect occupational exposure and to reflect new or revised employee positions with occupational exposure.

(v) The Exposure Control Plan shall be made available to the Assistant Secretary and the Director upon request for examination and copying.

(2) *Exposure determination.* (i) Each employer who has an employee(s) with occupational exposure as defined by paragraph (b) of this section shall prepare an exposure determination. This exposure determination shall contain the following:

(A) A list of all job classifications in which all employees in those job classifications have occupational exposure;

(B) A list of job classifications in which some employees have occupational exposure, and

(C) A list of all tasks and procedures or groups of closely related task and procedures in which occupational exposure occurs and that are performed by employees in job classifications listed in accordance with the provisions of paragraph (c)(2)(i)(B) of this standard.

(ii) This exposure determination shall be made without regard to the use of personal protective equipment.

(d) *Methods of compliance*—(1) *General*—Universal precautions shall be observed to prevent contact with blood or other potentially infectious materials. Under circumstances in which differentiation between body fluid types is difficult or impossible, all body fluids shall be considered potentially infectious materials.

(2) *Engineering and work practice controls.* (i) Engineering and work practice controls shall be used to eliminate or minimize employee exposure. Where occupational exposure remains after institution of these controls, personal protective equipment shall also be used.

(ii) Engineering controls shall be examined and maintained or replaced on a regular schedule to ensure their effectiveness.

(iii) Employers shall provide handwashing facilities which are readily accessible to employees.

(iv) When provision of handwashing facilities is not feasible, the employer shall provide either an appropriate antiseptic hand cleanser in conjunction with clean cloth/paper towels or antiseptic towelettes. When antiseptic hand cleansers or towelettes are used, hands shall be washed with soap and running water as soon as feasible.

(v) Employers shall ensure that employees wash their hands immediately or as soon as feasible after removal of gloves or other personal protective equipment.

(vi) Employers shall ensure that employees wash hands and any other skin with soap and water, or flush mucous membranes with water immediately or as soon as feasible following contact of such body areas with blood or other potentially infectious materials.

(vii) Contaminated needles and other contaminated sharps shall not be bent, recapped, or removed except as noted in paragraphs (d)(2)(vii)(A) and (d)(2)(vii)(B) below. Shearing or breaking of contaminated needles is prohibited.

(A) Contaminated needles and other contaminated sharps shall not be recapped or removed unless the employer can demonstrate that no alternative is feasible or that such action is required by a specific medical procedure.

(B) Such recapping or needle removal must be accomplished through the use of a mechanical device or a one-handed technique.

(viii) Immediately or as soon as possible after use, contaminated reusable sharps shall be placed in appropriate containers until properly reprocessed. These containers shall be:

(A) Puncture resistant;

(B) Labeled or color-coded in accordance with this standard;

(C) Leakproof on the sides and bottom; and

(D) In accordance with the requirements set forth in paragraph (d)(4)(ii)(E) for reusable sharps.

(ix) Eating, drinking, smoking, applying cosmetics or lip balm, ar l handling contact lenses are prohil ited in work areas where there is a reaso iable likelihood of occupational exposu e.

(x) Food and drink shall not be ept in refrigerators, freezers, shelves, ca inets or on countertops or benchtops wi ere blood or other potentially infectio s materials are present.

(xi) All procedures involving bl od or other potentially infectious materi ils shall be performed in such a mann er as to minimize splashing, spraying, spattering, and generation of drop ets of these substances.

(xii) Mouth pipetting/suctioning of blood or other potentially infectio s materials is prohibited.

(xiii) Specimens of blood or oth r potentially infectious materials sh ll be placed in a container which preve its leakage during collection, handlin , processing, storage, transport, or shipping.

(A) The container for storage, transport, or shipping shall be lab led or color-coded according to paragra h (g)(1)(i) and closed prior to being s ored, transported, or shipped. When a f cility utilizes Universal Precautions in t e handling of all specimens, the labe ling/ color-coding of specimens is not necessary provided containers are recognizable as containing specim ns. This exemption only applies while such specimens/containers remain with in the facility. Labeling or color-coding i accordance with paragraph (g)(1)( ) is required when such specimens/ containers leave the facility.

(B) If outside contamination of t e primary container occurs, the prim ary container shall be placed within a second container which prevents leakage during handling, processi g, storage, transport, or shipping and is labeled or color-coded according o the requirements of this standard.

(C) If the specimen could punct re the primary container, the primary co tainer shall be placed within a secondar container which is puncture-resist nt in addition to the above characterist cs.

(xiv) Equipment which may bec me contaminated with blood or other potentially infectious materials sh ll be examined prior to servicing or shi ping and shall be decontaminated as necessary, unless the employer ca n demonstrate that decontaminatio of such equipment or portions of suc equipment is not feasible.

(A) A readily observable label n accordance with paragraph (g)(1)( )(H) shall be attached to the equipmen stating which portions remain contaminated.

(B) The employer shall ensure that this information is conveyed to all affected employees, the servicing representative, and/or the manufacturer, as appropriate, prior to handling, servicing, or shipping so that appropriate precautions will be taken.

(3) Personal protective equipment—(i) Provision. When there is occupational exposure, the employer shall provide, at no cost to the employee, appropriate personal protective equipment such as, but not limited to, gloves, gowns, laboratory coats, face shields or masks and eye protection, and mouthpieces, resuscitation bags, pocket masks, or other ventilation devices. Personal protective equipment will be considered "appropriate" only if it does not permit blood or other potentially infectious materials to pass through to or reach the employee's work clothes, street clothes, undergarments, skin, eyes, mouth, or other mucous membranes under normal conditions of use and for the duration of time which the protective equipment will be used.

(ii) Use. The employer shall ensure that the employee uses appropriate personal protective equipment unless the employer shows that the employee temporarily and briefly declined to use personal protective equipment when, under rare and extraordinary circumstances, it was the employee's professional judgment that in the specific instance its use would have prevented the delivery of health care or public safety services or would have posed an increased hazard to the safety of the worker or co-worker. When the employee makes this judgement, the circumstances shall be investigated and documented in order to determine whether changes can be instituted to prevent such occurences in the future.

(iii) Accessibility. The employer shall ensure that appropriate personal protective equipment in the appropriate sizes is readily accessible at the worksite or is issued to employees. Hypoallergenic gloves, glove liners, powderless gloves, or other similar alternatives shall be readily accessible to those employees who are allergic to the gloves normally provided.

(iv) Cleaning, Laundering, and Disposal. The employer shall clean, launder, and dispose of personal protective equipment required by paragraphs (d) and (e) of this standard, at no cost to the employee.

(v) Repair and Replacement. The employer shall repair or replace personal protective equipment as needed to maintain its effectiveness, at no cost to the employee.

(vi) If a garment(s) is penetrated by blood or other potentially infectious materials, the garment(s) shall be removed immediately or as soon as feasible.

(vii) All personal protective equipment shall be removed prior to leaving the work area.

(viii) When personal protective equipment is removed it shall be placed in an appropriately designated area or container for storage, washing, decontamination or disposal.

(ix) Gloves. Gloves shall be worn when it can be reasonably anticipated that the employee may have hand contact with blood, other potentially infectious materials, mucous membranes, and non-intact skin; when performing vascular access procedures except as specified in paragraph (d)(3)(ix)(D); and when handling or touching contaminated items or surfaces.

(A) Disposable (single use) gloves such as surgical or examination gloves, shall be replaced as soon as practical when contaminated or as soon as feasible if they are torn, punctured, or when their ability to function as a barrier is compromised.

(B) Disposable (single use) gloves shall not be washed or decontaminated for re-use.

(C) Utility gloves may be decontaminated for re-use if the integrity of the glove is not compromised. However, they must be discarded if they are cracked, peeling, torn, punctured, or exhibit other signs of deterioration or when their ability to function as a barrier is compromised.

(D) If an employer in a volunteer blood donation center judges that routine gloving for all phlebotomies is not necessary then the employer shall:

(*1*) Periodically reevaluate this policy;

(*2*) Make gloves available to all employees who wish to use them for phlebotomy;

(*3*) Not discourage the use of gloves for phlebotomy; and

(*4*) Require that gloves be used for phlebotomy in the following circumstances:

(*i*) When the employee has cuts, scratches, or other breaks in his or her skin;

(*ii*) When the employee judges that hand contamination with blood may occur, for example, when performing phlebotomy on an uncooperative source individual; and

(*iii*) When the employee is receiving training in phlebotomy.

(x) Masks, Eye Protection, and Face Shields. Masks in combination with eye protection devices, such as goggles or glasses with solid side shields, or chin-length face shields, shall be worn whenever splashes, spray, spatter, or droplets of blood or other poten tially infectious materials may be gen erated and eye, nose, or mouth contam ination can be reasonably anticipated.

(xi) Gowns, Aprons, and Oth er Protective Body Clothing. Appr opriate protective clothing such as, but not limited to, gowns, aprons, lab c oats, clinic jackets, or similar outer g arments shall be worn in occupational e xposure situations. The type and charac teristics will depend upon the task and degree of exposure anticipated.

(xii) Surgical caps or hoods a nd/or shoe covers or boots shall be w orn in instances when gross contamin ation can reasonably be anticipated (e.g., autopsies, orthopaedic surgery).

(4) *Housekeeping.* (i) General Employers shall ensure that the worksite is maintained in a clean and sa nitary condition. The employer shall d etermine and implement an appropriate written schedule for cleaning and meth od of decontamination based upon th e location within the facility, type of surface to be cleaned, type of s oil present, and tasks or procedure s being performed in the area.

(ii) All equipment and enviro mental and working surfaces shall be c leaned and decontaminated after cont act with blood or other potentially infec tious materials.

(A) Contaminated work surfa ces be decontaminated with an app ropriate disinfectant after completion of procedures; immediately or as s oon as feasible when surfaces are ove rly contaminated or after any spill of blood or other potentially infectious m aterials; and at the end of the work shift if the surface may have become cont aminated since the last cleaning.

(B) Protective coverings, such as plastic wrap, aluminum foil, or imperviously-backed absorben paper used to cover equipment and environmental surfaces, shall b e removed and replaced as soon as feasible when they become ove rly contaminated or at the end of th e workshift if they may have bec ome contaminated during the shift.

(C) All bins, pails, cans, and similar receptacles intended for reuse which have a reasonable likelihood fo r becoming contaminated with bl ood or other potentially infectious mat erials shall be inspected and deconta minated on a regularly scheduled basis and cleaned and decontaminated immediately or as soon as feas ible upon visible contamination.

(D) Broken glassware which ma y contaminated shall not be picke d up directly with the hands. It shall be cleaned up using mechanical m eans.

such as a brush and dust pan, tongs, or forceps.

(E) Reusable sharps that are contaminated with blood or other potentially infectious materials shall not be stored or processed in a manner that requires employees to reach by hand into the containers where these sharps have been placed.

(iii) Regulated Waste.

(A) Contaminated Sharps Discarding and Containment. (*1*) Contaminated sharps shall be discarded immediately or as soon as feasible in containers that are:

(*i*) Closable;

(*ii*) Puncture resistant;

(*iii*) Leakproof on sides and bottom; and

(*iv*) Labeled or color-coded in accordance with paragraph (g)(1)(i) of this standard.

(*2*) During use, containers for contaminated sharps shall be:

(*i*) Easily accessible to personnel and located as close as is feasible to the immediate area where sharps are used or can be reasonably anticipated to be found (e.g., laundries);

(*ii*) Maintained upright throughout use; and

(*iii*) Replaced routinely and not be allowed to overfill.

(*3*) When moving containers of contaminated sharps from the area of use, the containers shall be:

(*i*) Closed immediately prior to removal or replacement to prevent spillage or protrusion of contents during handling, storage, transport, or shipping;

(*ii*) Placed in a secondary container if leakage is possible. The second container shall be:

(*A*) Closable;

(*B*) Constructed to contain all contents and prevent leakage during handling, storage, transport, or shipping; and

(*C*) Labeled or color-coded according to paragraph (*g*)(1)(*ii*) of this standard.

(*4*) Reusable containers shall not be opened, emptied, or cleaned manually or in any other manner which would expose employees to the risk of percutaneous injury.

(B) Other Regulated Waste Containment.(*1*) Regulated waste shall be placed in containers which are:

(*i*) Closable;

(*ii*) Constructed to contain all contents and prevent leakage of fluids during handling, storage, transport or shipping;

(*iii*) Labeled or color-coded in accordance with paragraph (g)(1)(i) this standard; and

(*iv*) Closed prior to removal to prevent spillage or protrusion of contents during handling, storage, transport, or shipping.

(*2*) If outside contamination of the regulated waste container occurs, it shall be placed in a second container. The second container shall be:

(*i*) Closable;

(*ii*) Constructed to contain all contents and prevent leakage of fluids during handling, storage, transport or shipping;

(*iii*) Labeled or color-coded in accordance with paragraph (g)(1)(i) of this standard; and

(*iv*) Closed prior to removal to prevent spillage or protrusion of contents during handling, storage, transport, or shipping.

(C) Disposal of all regulated waste shall be in accordance with applicable regulations of the United States, States and Territories, and political subdivisions of States and Territories.

(iv) Laundry.

(A) Contaminated laundry shall be handled as little as possible with a minimum of agitation. (1) Contaminated laundry shall be bagged or containerized at the location where it was used and shall not be sorted or rinsed in the location of use.

(2) Contaminated laundry shall be placed and transported in bags or containers labeled or color-coded in accordance with paragraph (g)(1)(i) of this standard. When a facility utilizes Universal Precautions in the handling of all soiled laundry, alternative labeling or color-coding is sufficient if it permits all employees to recognize the containers as requiring compliance with Universal Precautions.

(3) Whenever contaminated laundry is wet and presents a reasonable likelihood of soak-through of or leakage from the bag or container, the laundry shall be placed and transported in bags or containers which prevent soak-through and/or leakage of fluids to the exterior.

(B) The employer shall ensure that employees who have contact with contaminated laundry wear protective gloves and other appropriate personal protective equipment.

(C) When a facility ships contaminated laundry off-site to a second facility which does not utilize Universal Precautions in the handling of all laundry, the facility generating the contaminated laundry must place such laundry in bags or containers which are labeled or color-coded in accordance with paragraph (g)(1)(i).

(e) *HIV and HBV Research Laboratories and Production Facilities.* (1) This paragraph applies to research laboratories and production facilities engaged in the culture, production, concentration, experimentation, and manipulation of HIV and HBV. It does not apply to clinical or diagnostic laboratories engaged solely in the analysis of blood, tissues, or organs. These requirements apply in addition to the other requirements of the standard.

(2) Research laboratories and production facilities shall meet the following criteria:

(i) Standard microbiological practices. All regulated waste shall either be incinerated or decontaminated by a method such as autoclaving known to effectively destroy bloodborne pathogens.

(ii) Special practices.

(A) Laboratory doors shall be kept closed when work involving HIV or HBV is in progress.

(B) Contaminated materials that are to be decontaminated at a site away from the work area shall be placed in a durable, leakproof, labeled or color-coded container that is closed before being removed from the work area.

(C) Access to the work areas shall be limited to authorized persons. Written policies and procedures shall be established whereby only persons who have been advised of the potential biohazard, who meet any specific entry requirements, and who comply with all entry and exit procedures shall be allowed to enter the work areas and animal rooms.

(D) When other potentially infectious materials or infected animals are present in the work area or containment module, a hazard warning sign incorporating the universal biohazard symbol shall be posted on all access doors. The hazard warning sign shall comply with paragraph (g)(1)(ii) of this standard.

(E) All activities involving other potentially infectious materials shall be conducted in biological safety cabinets or other physical-containment devices within the containment module. No work with these other potentially infectious materials shall be conducted on the open bench.

(F) Laboratory coats, gowns, smocks, uniforms, or other appropriate protective clothing shall be used in the work area and animal rooms. Protective clothing shall not be worn outside of the work area and shall be decontaminated before being laundered.

(G) Special care shall be taken to avoid skin contact with other potentially infectious materials. Gloves shall be worn when handling infected animals and when making hand contact with other potentially infectious materials is unavoidable.

(H) Before disposal all waste from work areas and from animal rooms shall either be incinerated or decontaminated by a method such as autoclaving known to effectively destroy bloodborne pathogens.

(I) Vacuum lines shall be protected with liquid disinfectant traps and high-efficiency particulate air (HEPA) filters or filters of equivalent or superior efficiency and which are checked routinely and maintained or replaced as necessary.

(J) Hypodermic needles and syringes shall be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Only needle-locking syringes or disposable syringe-needle units (i.e., the needle is integral to the syringe) shall be used for the injection or aspiration of other potentially infectious materials. Extreme caution shall be used when handling needles and syringes. A needle shall not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The needle and syringe shall be promptly placed in a puncture-resistant container and autoclaved or decontaminated before reuse or disposal.

(K) All spills shall be immediately contained and cleaned up by appropriate professional staff or others properly trained and equipped to work with potentially concentrated infectious materials.

(L) A spill or accident that results in an exposure incident shall be immediately reported to the laboratory director or other responsible person.

(M) A biosafety manual shall be prepared or adopted and periodically reviewed and updated at least annually or more often if necessary. Personnel shall be advised of potential hazards, shall be required to read instructions on practices and procedures, and shall be required to follow them.

(iii) Containment equipment. (A) Certified biological safety cabinets (Class I, II, or III) or other appropriate combinations of personal protection or physical containment devices, such as special protective clothing, respirators, centrifuge safety cups, sealed centrifuge rotors, and containment caging for animals, shall be used for all activities with other potentially infectious materials that pose a threat of exposure to droplets, splashes, spills, or aerosols.

(B) Biological safety cabinets shall be certified when installed, whenever they are moved and at least annually.

(3) HIV and HBV research laboratories shall meet the following criteria:

(i) Each laboratory shall contain a facility for hand washing and an eye wash facility which is readily available within the work area.

(ii) An autoclave for decontamination of regulated waste shall be available.

(4) HIV and HBV production facilities shall meet the following criteria:

(i) The work areas shall be separated from areas that are open to unrestricted traffic flow within the building. Passage through two sets of doors shall be the basic requirement for entry into the work area from access corridors or other contiguous areas. Physical separation of the high-containment work area from access corridors or other areas or activities may also be provided by a double-doored clothes-change room (showers may be included), airlock, or other access facility that requires passing through two sets of doors before entering the work area.

(ii) The surfaces of doors, walls, floors and ceilings in the work area shall be water resistant so that they can be easily cleaned. Penetrations in these surfaces shall be sealed or capable of being sealed to facilitate decontamination.

(iii) Each work area shall contain a sink for washing hands and a readily available eye wash facility. The sink shall be foot, elbow, or automatically operated and shall be located near the exit door of the work area.

(iv) Access doors to the work area or containment module shall be self-closing.

(v) An autoclave for decontamination of regulated waste shall be available within or as near as possible to the work area.

(vi) A ducted exhaust-air ventilation system shall be provided. This system shall create directional airflow that draws air into the work area through the entry area. The exhaust air shall not be recirculated to any other area of the building, shall be discharged to the outside, and shall be dispersed away from occupied areas and air intakes. The proper direction of the airflow shall be verified (i.e., into the work area).

(5) *Training Requirements.* Additional training requirements for employees in HIV and HBV research laboratories and HIV and HBV production facilities are specified in paragraph (g)(2)(ix).

(f) *Hepatitis B vaccination and post-exposure evaluation and follow-up—(1) General.* (i) The employer shall make available the hepatitis B vaccine and vaccination series to all employees who have occupational exposure, and post-exposure evaluation and follow-up to all employees who have had an exposure incident.

(ii) The employer shall ensure that all medical evaluations and procedures including the hepatitis B vaccine and vaccination series and post-exposure evaluation and follow-up, including prophylaxis, are:

(A) Made available at no cost to the employee;

(B) Made available to the employee at a reasonable time and place;

(C) Performed by or under the supervision of a licensed physician or by or under the supervision of another licensed healthcare professional; and

(D) Provided according to recommendations of the U.S. Public Health Service current at the time these evaluations and procedures take place, except as specified by this paragraph (f).

(iii) The employer shall ensure that all laboratory tests are conducted by an accredited laboratory at no cost to the employee.

(2) *Hepatitis B Vaccination.* (i) Hepatitis B vaccination shall be made available after the employee has received the training required in paragraph (g)(2)(vii)(I) and within 10 working days of initial assignment to all employees who have occupational exposure unless the employee has previously received the complete hepatitis B vaccination series, antibody testing has revealed that the employee is immune, or the vaccine is contraindicated for medical reasons.

(ii) The employer shall not make participation in a prescreening program a prerequisite for receiving hepatitis B vaccination.

(iii) If the employee initially declines hepatitis B vaccination but at a later date while still covered under the standard decides to accept the vaccination, the employer shall make available hepatitis B vaccination at that time.

(iv) The employer shall assure that employees who decline to accept hepatitis B vaccination offered by the employer sign the statement in appendix A.

(v) If a routine booster dose(s) of hepatitis B vaccine is recommended by the U.S. Public Health Service at a future date, such booster dose(s) shall be made available in accordance with section (f)(1)(ii).

(3) *Post-exposure Evaluation and Follow-up.* Following a report of an exposure incident, the employer shall make immediately available to the exposed employee a confidential medical evaluation and follow-up, including at least the following elements:

(i) Documentation of the route(s) of exposure, and the circumstances under which the exposure incident occurred;

(ii) Identification and documentation of the source individual, unless the employer can establish that identification is infeasible or prohibited by state or local law;

(A) The source individual's blood shall be tested as soon as feasible and

after consent is obtained in order to determine HBV and HIV infectivity. If consent is not obtained, the employer shall establish that legally required consent cannot be obtained. When the source individual's consent is not required by law, the source individual's blood, if available, shall be tested and the results documented.

(B) When the source individual is already known to be infected with HBV or HIV, testing for the source individual's known HBV or HIV status need not be repeated.

(C) Results of the source individual's testing shall be made available to the exposed employee, and the employee shall be informed of applicable laws and regulations concerning disclosure of the identity and infectious status of the source individual.

(iii) Collection and testing of blood for HBV and HIV serological status:

(A) The exposed employee's blood shall be collected as soon as feasible and tested after consent is obtained.

(B) If the employee consents to baseline blood collection, but does not give consent at that time for HIV serologic testing, the sample shall be preserved for at least 90 days. If, within 90 days of the exposure incident, the employee elects to have the baseline sample tested, such testing shall be done as soon as feasible.

(iv) Post-exposure prophylaxis, when medically indicated, as recommended by the U.S. Public Health Service;

(v) Counseling; and

(vi) Evaluation of reported illnesses.

(4) *Information Provided to the Healthcare Professional.* (i) The employer shall ensure that the healthcare professional responsible for the employee's Hepatitis B vaccination is provided a copy of this regulation.

(ii) The employer shall ensure that the healthcare professional evaluating an employee after an exposure incident is provided the following information:

(A) A copy of this regulation;

(B) A description of the exposed employee's duties as they relate to the exposure incident;

(C) Documentation of the route(s) of exposure and circumstances under which exposure occurred;

(D) Results of the source individual's blood testing, if available; and

(E) All medical records relevant to the appropriate treatment of the employee including vaccination status which are the employer's responsibility to maintain.

(5) *Healthcare Professional's Written Opinion.* The employer shall obtain and provide the employee with a copy of the evaluating healthcare professional's written opinion within 15 days of the completion of the evaluation.

(i) The healthcare professional's written opinion for Hepatitis B vaccination shall be limited to whether Hepatitis B vaccination is indicated for an employee, and if the employee has received such vaccination.

(ii) The healthcare professional's written opinion for post-exposure evaluation and follow-up shall be limited to the following information:

(A) That the employee has been informed of the results of the evaluation; and

(B) That the employee has been told about any medical conditions resulting from exposure to blood or other potentially infectious materials which require further evaluation or treatment.

(iii) All other findings or diagnoses shall remain confidential and shall not be included in the written report.

(6) *Medical recordkeeping.* Medical records required by this standard shall be maintained in accordance with paragraph (h)(1) of this section.

(g) *Communication of hazards to employees*— (1) *Labels and signs.* (i) Labels. (A) Warning labels shall be affixed to containers of regulated waste, refrigerators and freezers containing blood or other potentially infectious material; and other containers used to store, transport or ship blood or other potentially infectious materials, except as provided in paragraph (g)(1)(i)(E), (F) and (G).

(B) Labels required by this section shall include the following legend:

BIOHAZARD

BIOHAZARD

(C) These labels shall be fluorescent orange or orange-red or predominantly so, with lettering or symbols in a contrasting color.

(D) Labels required by affixed as close as feasible to the container by string, wire, adhesive, or other method that prevents their loss or unintentional removal.

(E) Red bags or red containers may be substituted for labels.

(F) Containers of blood, blood components, or blood products that are labeled as to their contents and have been released for transfusion or other clinical use are exempted from the labeling requirements of paragraph (g).

(G) Individual containers of blood or other potentially infectious materials that are placed in a labeled container during storage, transport, shipment or disposal are exempted from the labeling requirement.

(H) Labels required for contaminated equipment shall be in accordance with this paragraph and shall also state which portions of the equipment remain contaminated.

(I) Regulated waste that has been decontaminated need not be labeled or color-coded.

(ii) Signs. (A) The employer shall post signs at the entrance to work areas specified in paragraph (e), HIV and HBV Research Laboratory and Production Facilities, which shall bear the following legend:

BIOHAZARD

BIOHAZARD

(Name of the Infectious Agent)
(Special requirements for entering the area)
(Name, telephone number of the laboratory director or other responsible person.)

(B) These signs shall be fluorescent orange-red or predominantly so, with lettering or symbols in a contrasting color.

(2) *Information and Training.* (i) Employers shall ensure that all employees with occupational exposure participate in a training program which must be provided at no cost to the employee and during working hours.

(ii) Training shall be provided as follows:

(A) At the time of initial assignment to tasks where occupational exposure may take place;

(B) Within 90 days after the effective date of the standard; and

(C) At least annually thereafter.

(iii) For employees who have received training on bloodborne pathogens in the year preceding the effective date of the standard, only training with respect to the provisions of the standard which were not included need be provided.

(iv) Annual training for all employees shall be provided within one year of their previous training.

(v) Employers shall provide additional training when changes such as modification of tasks or procedures or institution of new tasks or procedures affect the employee's occupational exposure. The additional training may be limited to addressing the new exposures created.

(vi) Material appropriate in content and vocabulary to educational level, literacy, and language of employees shall be used.

(vii) The training program shall contain at a minimum the following elements:

(A) Anaccessible copy of the regulatory text of this standard and an explanation of its contents;

(B) A general explanation of the epidemiology and symptoms of bloodborne diseases;

(C) An explanation of the modes of transmission of bloodborne pathogens;

(D) An explanation of the employer's exposure control plan and the means by which the employee can obtain a copy of the written plan;

(E) An explanation of the appropriate methods for recognizing tasks and other activities that may involve exposure to blood and other potentially infectious materials;

(F) An explanation of the use and limitations of methods that will prevent or reduce exposure including appropriate engineering controls, work practices, and personal protective equipment;

(G) Information on the types, proper use, location, removal, handling, decontamination and disposal of personal protective equipment;

(H) An explanation of the basis for selection of personal protective equipment;

(I) Information on the hepatitis B vaccine, including information on its efficacy, safety, method of administration, the benefits of being vaccinated, and that the vaccine and vaccination will be offered free of charge;

(J) Information on the appropriate actions to take and persons to contact in an emergency involving blood or other potentially infectious materials;

(K) An explanation of the procedure to follow if an exposure incident occurs, including the method of reporting the incident and the medical follow-up that will be made available;

(L) Information on the post-exposure evaluation and follow-up that the employer is required to provide for the employee following an exposure incident;

(M) An explanation of the signs and labels and/or color coding required by paragraph (g)(1); and

(N) An opportunity for interactive questions and answers with the person conducting the training session.

(viii) The person conducting the training shall be knowledgeable in the subject matter covered by the elements contained in the training program as it relates to the workplace that the training will address.

(ix) Additional Initial Training for Employees in HIV and HBV Laboratories and Production Facilities. Employees in HIV or HBV research laboratories and HIV or HBV production facilities shall receive the following initial training in addition to the above training requirements.

(A) The employer shall assure that employees demonstrate proficiency in standard microbiological practices and techniques and in the practices and operations specific to the facility before being allowed to work with HIV or HBV.

(B) The employer shall assure that employees have prior experience in the handling of human pathogens or tissue cultures before working with HIV or HBV.

(C) The employer shall provide a training program to employees who have no prior experience in handling human pathogens. Initial work activities shall not include the handling of infectious agents. A progression of work activities shall be assigned as techniques are learned and proficiency is developed. The employer shall assure that employees participate in work activities involving infectious agents only after proficiency has been demonstrated.

(h) *Recordkeeping—(1) Medical Records.* (i) The employer shall establish and maintain an accurate record for each employee with occupational exposure, in accordance with 29 CFR 1910.20.

(ii) This record shall include:

(A) The name and social security number of the employee;

(B) A copy of the employee's hepatitis B vaccination status including the dates of all the hepatitis B vaccinations and any medical records relative to the employee's ability to receive vaccination as required by paragraph (f)(2);

(C) A copy of all results of examinations, medical testing, and follow-up procedures as required by paragraph (f)(3);

(D) The employer's copy of the healthcare professional's written opinion as required by paragraph (f)(5); and

(E) A copy of the information provided to the healthcare professional as required by paragraphs (f)(4)(ii)(B)(C) and (D).

(iii) Confidentiality. The employer shall ensure that employee medical records required by paragraph (h)

(A) Kept confidential; and

(B) Are not disclosed or reported without the employee's express written consent to any person within or outside the workplace except as required by this section or as may be required by law.

(iv) The employer shall maintain the records required by paragraph (h) for at least the duration of employment plus 30 years in accordance with 29 CFR 1910.20.

(2) *Training Records. (i) Training records shall include the following information:*

(A) The dates of the training sessions;

(B) The contents or a summary of the training sessions;

(C) The names and qualifications of persons conducting the training; and

(D) The names and job titles of all persons attending the training sessions.

(ii) Training records shall be maintained for 3 years from the date on which the training occurred.

(3) *Availability.* (i) The employer shall ensure that all records required to be maintained by this section shall be made available upon request to the Assistant Secretary and the Director for examination and copying.

(ii) Employee training records required by this paragraph shall be provided upon request for examination and copying to employees, to employee representatives, to the Director, and to the Assistant Secretary in accordance with 29 CFR 1910.20.

(iii) Employee medical records required by this paragraph shall be provided upon request for examination and copying to the subject employee, to anyone having written consent of the subject employee, to the Director, and the Assistant Secretary in accordance with 29 CFR 1910.20.

(4) *Transfer of Records.* (i) The employer shall comply with the requirements involving transfer of records set forth in 29 CFR 1910.20(h).

(ii) If the employer ceases to do business and there is no successor employer to receive and retain the records for the prescribed period, the employer shall notify the Director, at least three months prior to their disposal and transmit them to the Director, if required by the Director to do so, within that three month period.

(i) *Dates—(1) Effective Date.* The standard shall become effective on March 6, 1992.

(2) The Exposure Control Plan required by paragraph (c)(1) of this section shall be completed on or before May 5, 1992.

(3) Paragraph (g)(2) Information and Training and (h) Recordkeeping shall take effect on or before June 4, 1992.

(4) Paragraphs (d)(2) Engineering and Work Practice Controls..(d)(3) Personal Protective Equipment, (d)(4) Housekeeping, (e) HIV and HBV Research Laboratories and Production Facilities, (f) Hepatitis B Vaccination and Post-Exposure Evaluation and Follow-up, and (g) (1) Labels and Signs, shall take effect July 6, 1992.

Appendix A to Section 1910.1030—Hepatitis B Vaccine Declination (Mandatory)

I understand that due to my occupational exposure to blood or other potentially infectious materials I may be at risk of acquiring hepatitis B virus (HBV) infection. I have been given the opportunity to be vaccinated with hepatitis B vaccine, at no charge to myself. However, I decline hepatitis B vaccination at this time. I understand that by declining this vaccine, I continue to be at risk of acquiring hepatitis B, a serious disease. If in the future I continue to have occupational exposure to blood or other potentially infectious materials and I want to be vaccinated with hepatitis B vaccine, can receive the vaccination series at no charge to me.

[FR Doc. 91-28886 Filed 12-2-91; 8:45 am]
BILLING CODE 4510-26-M

DELTA
Materials for center to have at time of training:
6/7/93

For safety

1. Institutional OSHA training policy, institutional procedures

2. Appropriate safety supplies for a phlebotomy station. OSHA acceptable personal protective equipment, appropriate disposal units for sharps and regulated medical waste, supplies for cleaning spills, etc.)

For Cholestech operation

1. Cholestech instrument, printer, paper, Optic check cassette, manual.
   Materials for fingerstick blood draw (lancets, alcohol swabs, etc.)
   Practice cassettes

2. Copy of procedure (supplied by R. Reed)

For Baseline samples.

1. Phlebotomy supplies for drawing 4 Vacutainers - Vacutainer adapter and Vacutainers.
   Vacutainers: 2 6-mL SST tubes (Becton Dickinson 367784)
   1 3-ml lavender top (EDTA) (Becton-Dickinson 367661)
   1 10 ml lavender top (EDTA) (Becton-Dickinson 6457)

2. Clinical centrifuge, transfer pipets, supplies. (Are also needed for endpoint samples. See attachments describing supplies for endpoint samples)

3. 2 Amber cryovials (0.5 ml) for buffy coats (e.g. Sarstedt 72.730.008)

Materials for center to have at time of training (cont):
6/7/93

For endpoint samples

1. Blood collection tray - Supplies for drawing 5 Vacutainers. (See Chapter 10, p. 11, attached - Blood Collection Rack and Blood collection tray checklist)
   Vacutainers: 2 9.5 mL SST (Becton-Dickinson 6510)
   2 4.5 ml blue top (citrate) (Becton-Dickinson 6418)
   1 10 ml lavender top (EDTA) (Becton-Dickinson 6457)

2. Equipment and supplies for sample processing and aliquoting including:

   a. Two centrifuges (one refrigerated and one room temperature) with holders for Vacutainers and capable of at least 1500xg (preferably 3000xg).

   b. 500 ul MLA pipet

   c. Other supplies and equipment as listed from Chapter 10 (attached)

3. Supplies for archiving and shipping samples

   For each subject endpoint sample

   28 0.5 ml cryovials (Sarstedt #72.730.003) without caps

   14 red caps - Sarstedt #65.716.003
   4 green caps - Sarstedt #65.716.005
   4 blue caps - Sarstedt #65.716.001
   6 lavender caps - Sarstedt #65.716.008

L. Sample Processing Checklist:

[redacted]

[redacted]

Pipette: MLA 500

Pipette tips

Pasteur pipette with bulbs

Ice bath/ice

Latex gloves

Lab mat

Kimwipes

Biohazard waste bags

Clorox (10% in wash bottle)

Pen/pencils

Study participant aliquot racks (2) with prelabeled aliquots for cryovials

Phlebotomy/Processing form (page 1 completed)

Freezer

Refrigerator

Centrifuges -- room temperature and 4°C

Balance tube

Thermometer

Zip-lock bag

Lab coats, gloves, face shields

Blood collection tray checklist per tray:

- 10 - 21 gauge butterfly with lure adaptors
- 10 - alcohol swabs
- 15 - Band-Aids
- 15 - gauze pads
- 5 - Vacutainer holders
- 2 - tourniquets
- 1 - smelling salts
- 1 - timer or stop watch
- 2 - pencil/pen
- - Latex gloves
- 1 - hemostat
- 1 - adhesive tape
- 1 - scissors

Approximately 10 minutes before draw:

- 1 - styrofoam ice bath filled with ice.

Optional:

- 10 cc plastic syringes
- 20 cc plastic syringes
- needles for syringes

Per participant:

- 1 - blood tube rack with four (or five) draw tubes (labeled and numbered) (four = baseline; five = endpoint)
- 1 - phlebotomy/processing form

Have available on phlebotomy cart:

- basin
- cold cloth
- tube mixer
- biohazard containers
- needle/sharps container
- paper towels

2. Blood Collection Rack.

The collection tray itself is made of hard plastic which is unbreakable and can be easily cleaned. The tray has 10 individual compartments which will be filled with the following supplies:

21 g butterfly needles with luer adapter
alcohol swabs
Band-Aids
Gauze
Tourniquets (2)
Vacutainer holders
Needle/Sharps container
Smelling salts
Timer/Stop watch
Scissors
Adhesive tape
Styrofoam ice bath filled approximately 10 minutes before draw
Pencils/Pens
Latex gloves
Test tube racks (2)

A separate rack contains the necessary draw tubes, etc. for each participant. The tubes are arranged according to the priority of the draw. This rack will fit into the blood collection tray. The blood collection tubes are pre-labeled with identification numbers.

Delta Project
Laboratory Precision Evaluation

Instructions for Analysis

Enclosed are CDC frozen reference sera to be analyzed by your laboratory to estimate with-in laboratory precision for total and HDL-cholesterol measurement. All samples must be kept frozen until the time of analysis (preferably at -60°C or lower). **Carefully read these instructions before unpacking the samples from the boxes and beginning the analyses.**

You have received 2 boxes of samples, one labeled HDL and another labeled TC. The box labeled TC contains 9 vials, three vials each of three different concentrations. The box labeled HDL contains 18 vials, two vials each of three different concentrations. The vials are packed according to run numbers which are indicated on the inside flap of each box. Use care to maintain the arrangement and not mix the vials of the different runs.

Analysis Scheme:

TOTAL CHOLESTEROL

1. The CDC samples should be analyzed as part of a routine run of patient specimens. Do not analyze the CDC samples in a special run. One vial of each level should be analyzed in quadruplicate in each of three runs. Only one run should be performed per day.

2. On the day of the run, remove the 3 vials for the appropriate run. Immediately place the box with the unused vials back in the freezer. Allow the vials to thaw at room temperature or in a 25°C water bath for 30 minutes. Mix the serum by gentle inversion 2-3 times during this period. Just before pipetting, mix the sample again by gentle and thorough swirling or on a blood mixing wheel or a similar device for five minutes. Do not vortex or shake the serum vigorously. Be certain that the serum appears homogeneous before sampling.

3. Take four aliquots from each vial and analyze for total cholesterol. The four aliquots should be treated the same as four independent patient specimens and should be randomly distributed throughout the other patient specimens in the run.

4. Record the results on the forms provided. Be certain to correctly transcribe the 6 digit code from each CDC vial to the data form under "TC Vial #".

Instructions for Analysis (continued):

HDL-CHOLESTEROL

1. The CDC samples should be analyzed as part of a routine run of patient specimens. Do not analyze the CDC samples in a special run. Two vials of each level should be analyzed in duplicate in each of three runs. Only one run should be performed per day.

2. On the day of the run, remove the 6 vials for the appropriate run. Immediately place the box with the unused vials back in the freezer. Allow the vials to thaw at room temperature or in a 25°C water bath for 30 minutes. Mix the serum by gentle inversion 2-3 times during this period. Just before pipetting, mix the sample again by gentle and thorough swirling or on a blood mixing wheel or a similar device for five minutes. Do not vortex or shake the serum vigorously. Be certain that the serum appears homogeneous before sampling.

3. Take two aliquots from each vial and analyze for HDL-cholesterol. The aliquots should be treated the same as independent patient specimens and should be randomly distributed throughout the other patient specimens in the run.

4. Record the results on the forms provided. Be certain to correctly transcribe the 6 digit code from each CDC vial to the data form under "HDL Vial #".

Delta Project
Laboratory Precision Evaluation

Total Cholesterol

Laboratory Name: _____

Run 1	Date: _____			
TC Vial #	Results			

Run 2	Date: _____			
TC Vial #	Results			

Run 3	Date: _____			
TC Vial #	Results			

Return this form to: Gary L. Myers FAX: (404) 488-4609
Copy to: Paul Stewart FAX: (919) 966-7141

Delta Project
Laboratory Precision Evaluation

HDL - Cholesterol

Laboratory Name: _____

Run 1	Date: _____	
HDL Vial #	Results	

Run 2	Date: _____	
HDL Vial #	Results	

Run 3	Date: _____	
HDL Vial #	Results	

Return this form to: Gary L. Myers FAX: (404) 488-4609
Copy to: Paul Stewart FAX: (919) 966-7141

DELTA PROJECT – TOTAL CHOLESTEROL PRECISION

within laboratory

CV-total

lab	pool 66 mean	CV	pool 67 mean	CV	pool 71 mean	CV
colu	183	0.7	220	0.9	274	0.6
loui	178	0.9	216	1.0	263	0.7
mibh	182	1.3	219	1.2	274	1.2
minn	175	1.9	210	1.6	257	1.6
stan	184	0.4	220	0.6	272	0.5
	180.4	1.1	217.0	1.1	268.0	0.9
CDC Mean	183		220		273	

DELTA PROJECT – HDL PRECISION

within laboratory

CV-total

lab	pool 36 mean	CV	pool 81 mean	CV	pool 88 mean	CV
colu	60	0.9	40	1.3	27	1.9
loui	56	1.9	38	1.4	27	2.1
mibh	60	2.0	40	2.0	28	2.2
minn	53	1.5	36	1.3	25	1.3
stan	60	1.1	41	0.8	29	0.9
	57.8	1.5	39.0	1.4	27.2	1.7
CDC mean	59.0		39.6		27.2	

DELTA PROJECT – TOTAL CHOLESTEROL PRECISION

	POOL 66 VARIANCE	CV	% TOTAL	POOL 67 VARIANCE	CV	% TOTAL	POOL 71 VARIANCE	CV	% TOTAL	MEAN CV	MEAN % TOTAL
TOTAL	17.1	2.3	100	22.2	2.2	100	63.3	3.0	100	2.5	100
LAB	12.1	1.9	71	15.3	1.8	69	55.3	2.8	87	2.2	75.7
RUN	2.4	0.9	14	4.5	1.0	20	3.1	0.7	5	0.8	13.0
REPS.	2.6	0.9	15	2.5	0.7	11	4.9	0.8	8	0.8	11.3
MEAN	180.26			217.1			268.4				

DELTA PROJECT – HDL PRECISION

	POOL 36 VARIANCE	CV	% TOTAL	POOL 81 VARIANCE	CV	% TOTAL	POOL 88 VARIANCE	CV	% TOTAL	MEAN CV	MEAN % TOTAL
TOTAL	1.4	5.9	100	4.1	5.2	100	3.0	6.3	100	5.8	100
LAB	.6	5.6	93	3.8	5.0	92	2.7	6.1	92	5.6	92.3
RUN	.2	0.7	1	0.1	0.7	2	0.0	0.6	1	0.7	1.3
REPS.	0.7	1.4	6	0.2	1.3	6	0.2	1.7	7	1.5	6.3
MEAN	57.7			38.7			27.2				

Variance components shown above are total, among labs, among runs-within labs, and among replicates.

Analysis of precision data for delta project:

An analysis of variance of the reported concentrations from laboratories was done to study the source of variability. For TC, 75% of total variation was due to among-lab differences, and this was 92% for HDL. The within-lab CV averages about 1% for TC and 1.5% for HDL for these laboratories.

For TC, the Minn lab had the highest CV for TC (~1.7%), while the Mibh lab had the highest CV for HDL (~2.1%). The Stan lab had the best precision for both TC and HDL.

APPENDIX A

INFORMED CONSENT ISSUES

A.1 INFORMED CONSENT ISSUES

A.1 Reasons for Problems in Obtaining Informed Consent

Many trials are unable to achieve their recruitment goals because the investigators fail to obtain informed consent from patients who pass (or would pass) screening examinations. This failure may occur for the following reasons.

1. Culture and tradition of the country, or the investigator's own background, make it emotionally difficult for the investigator to ask patients for their informed consent. The easiest way to resolve this problem is to have someone else ask the patients. This person should be more comfortable with the process and could be another investigator, a nurse, the trial coordinator, or a special resource person (e.g., a translator). For DELTA, it is necessary to limit participants to those who can read and understand English.

2. The investigator who asks for informed consent may be rather stiff or brusque in describing the trial and in asking for the patient's informed consent. In this case, the investigator should spend more time with each patient and not rush the process.

GUIDELINES FOR OBTAINED INFORMED CONSENT

(Taken from Spilker, Bert and Cramer, Joyce A., *Patient Recruitment in Clinical Trials*. New York: Raven Press, 1992.)

A.2 OFFERING PAYMENTS TO RECRUIT VOLUNTEERS OR PATIENTS

A.2.1 Offering Money to Recruit Volunteers

Because volunteers receive no medical benefit from participation in a dietary trial, and are often subjected to severe restrictions in daily living, it is reasonable to pay them for their participation. This is standard practice by many academicians and is accepted as both ethical and appropriate. Money should not be given only to those volunteers who complete an entire trial because this places undue pressure on volunteers to remain in a trial from which they might rather withdraw. Money given to volunteers should be prorated based on the extent of their participation unless there are legal requirements to give all volunteers the entire sum, even for partial participation. The amount of money offered cannot be so excessive that volunteers would feel strong pressure to remain in a clinical trial from which they would rather withdraw.

A.2.2 Basing Payments on Reasons for Leaving a Trial

Subjects may leave a diet study for several reasons. The rigid control of diet over an extended period places severe constraints on daily living. Subjects have given over control of a significant part of their lives to the investigator and study personnel. Inability to comply with these restrictions may make them ineligible to continue with the study. Categories of noncompliance include willful departures from the study diet, severe illness, extended absences for business or personal emergencies. The decision to withdraw a subject involuntarily from the study is made by the Principal Investigator after discussion with the DELTA Steering Committee. Payment to the subject for partial participation is at the discretion of the local field center. If a subject withdraws voluntarily from the study, it is important for the investigator to determine if and to what extent the diet contributed to the dropout. If any link, however remote, is found with the treatment, the situation should be categorized as diet related. Such instances would require full payment to the subject, who provided important data on adverse effects leading to discontinuation of the diet. Not only should the patient or volunteer not feel that he or she disappointed the investigator by stopping the protocol, but each person should be encouraged to report his/her reasons for leaving the study as fully as possible. Subjects who feel vaguely uncomfortable should be encouraged to express this effect rather than deny any problem when asking to withdraw.

Although a subject may not have completed a study for personal reasons, he or she should be given partial payment based on the proportion of the protocol fulfilled. It would be unfair not to provide compensation for activities that might have taken time and effort even if the data cannot be used in study outcome analyses.

A.3 USING COERCIVE TACTICS TO RECRUIT PATIENTS

Any time there is a superior-subservient relationship between two individuals, it is impossible to ensure that coercion was not felt by the person in the subservient position, even if coercion was not overtly used in the recruitment process. Three examples of this relationship in clinical research are (1) an investigator recruiting his or her staff or students, (2) a professor recruiting his or her students, and (3) a pharmaceutical company conducting a clinical trial and recruiting its junior level employees.

Such groups cannot provide a true informed consent if the volunteers believe that refusal to enroll may result in being fired, may affect their career prospects, or influence their course grades.

A.4 ETHICAL GUIDELINES FOR RECRUITMENT OF HEALTHY VOLUNTEERS

1. Use of an informed consent that includes standard elements. The most important element that differs between the vast majority of volunteers and most patients involves the statement that the research is unlikely to be of any direct personal benefit to the volunteer. Similarly, some patients are unlikely to benefit from their participation in a trial.

2. Normal volunteers should not be enrolled in clinical trials where there is perceptible risk. The definition and application of the term risk raises numerous other issues (e.g., how is perceptible risk measured and how much risk is excessive). The balance should be determined by considering the benefits to be gained by society as a whole versus the risks to the individual.

3. Volunteers should not be sought where a dependency relationship exists.

4. Volunteers sought from any organized group should be contacted after officials of that organization have been informed and have fully discussed the issues with those who will conduct the trial.

5. Self-experimentation or experimentation on colleagues or friends of the investigator should be subjected to the same professional and ethical reviews as for other more conventional clinical trials.

6. Issues of liability for injury should be considered and discussed for both negligent causes. It should be determined who is responsible for payment and how payment would be determined. Numerous options and proposals exist in this area (e.g., see Macrae et. al., 1989).

IRB approval date _____
Approval expiration date_____

Columbia Presbyterian Medical Center

Consent to participate in a Research Study:

The purpose of this consent form is to provide you with the information you need to consider in deciding whether to participate in this research study.

Study title: "The effects of dietary fats on lipid metabolism and hemostasis. Protocol 1. Varying levels of saturated fats in healthy individuals.

IRB # 6065

Principal Investigator: Henry N. Ginsberg, Professor of Medicine

Study Purpose:

You are invited to participate in a research study of the effects of eating saturated fat on your blood cholesterol and fat levels and on the clotting activity of your blood. Dr. Ginsberg and his associates hope to learn more about the way the dietary fat affects the amount of clots. All of this information is important because of the link between diet, blood cholesterol, blood clotting, and the chance of having a heart attack or stroke. You were selected as a possible participant in this study because you are in good health and have average cholesterol levels. In this study, we will be comparing the effects of diets containing different amounts of saturated fat (the type of fat this raises blood cholesterol): one diet will have the amount of saturated fat that is usually eaten by Americans (15% of total calories), one will have the amount recommended by the American Heart Associate for all Americans (9% of total calories) and one will contain a lower level that is being suggested as optimal for Americans to eat (5% of total calories). The diets will all contain the same amounts of protein, monounsaturated fat, polyunsaturated fat and cholesterol. The reductions in calories from saturated fats will be matched by increases in calories from carbohydrates.

The idea behind this study is that reducing the amount of saturated fat in the diet will reduce the level of cholesterol and fat in the blood, and will make the blood less likely to clot. Heart attacks are the major cause of death in the United States, and we know that both the level of cholesterol and fat in the blood as well as the likelihood that the blood will clot contribute to an individual's risk for developing coronary artery disease (narrowing of the blood vessel supplying the heart with oxygen). Coronary artery disease leads to heart attacks. Although many studies have looked at the relationship between saturated fat in the diet and the level of cholesterol and fat in the blood, we still have much to learn. Specifically, this study will focus on females along with males, on African Americans along with other racial groups, and on a pre and postmenopausal females. This study will also look at how blood clotting might be affected by diet; very few studies have addressed this question.

Study Procedures:

If you decide to participate, you will agree to eat only foods that we provide for you (except for one dinner on the weekend) during three 8-week periods. You will come to the Bard Hall cafeteria for lunch and dinner and eat your meals there Monday through Friday. We will give you packed snacks and breakfasts to eat at home. On Friday evenings you will be given packages containing weekend breakfasts, lunches, one dinner and snacks. There will be breaks of 4-6 weeks between the first and second, and between the second and third diet periods.

We will weigh you twice weekly and you will provide the staff dieticians with information about your one weekend meal as well as alcohol intake (limited to five drinks per week) and any non-study foods you may have eaten.

During the final four weeks of the study (weeks 5,6,7,8) you will come to our offices on one day of each week for blood tests. All blood samples will be obtained after a 12 hour overnight fast.

The amount of blood drawn at a single visit will be about one ounce (two tablespoons). Each visit for blood sampling should take no more than 20 minutes.

Successful completion of this study depends on the excellent cooperation of the participants. If, during the study, you cannot eat the foods provided and/or eat other foods, you will be asked to leave the study.

You may agree to participate in a part of the study specifically looking at your ability to notice what and how much food you eat each day. This part of the study will require you to take part in 15-20 minute telephone conversations on 12 occasions during the study. If you do not wish to participate in this particular part of the study, it will not affect your overall participation.

Study risks:

The diets to be used in these studies are eaten by significant numbers of typical Americans. We do not see any risks associated with consumption of these diets.

The risks involved with blood drawing include some local pain and bruising from venipuncture: we will use well trained and experienced phlebotomists. Blood sampling can also cause light-headedness and dizziness: we will watch for this and if it occurs we can alleviate symptoms by having the subject lie flat with feet raised.

Alternatives:

As this is not a treatment, there is no alternative other than not participating.

Compensation:

All foods, meals, snacks, fluids will be provided at no cost to participants. In some cases subjects will be reimbursed for travel/parking fees.

Confidentiality:

Patient files and results will all be coded. All data will be locked in a file cabinet by the Principal Investigator.

Participation is voluntary:

Your participation in this study is completely voluntary. You can refuse to participate or withdraw from the study at any time, and such a decision will not affect your medical care, employment, or student status at Columbia Presbyterian Medical Center now or in the future. As noted above, the investigators may ask you to leave the study if you cannot comply completely with the diets.

Questions:

If you have any questions, please ask. In the future, should you have any questions you can reach Dr. Berglund or Dr. Ginsberg at 305-3741 and they will do their best to answer them. If you have any questions on your rights as a research subject you can call the Institutional Review Board (212-305-5883) for information.

Signature: /Date

(Investigator)

Consent to Participate in the Study

I have discussed this study with Dr. Ginsberg (or his designated associate) to my satisfaction. I understand that my participation is voluntary and that I can withdraw from the study at any time without prejudice. I have read the above and agree to enter this research study. Signing this form does not waive any of my legal rights.

I have been informed that if I believe that I have sustained injury as a result of participating in a research study, I may contact the Principal Investigators, Henry N. Ginsberg at 305-3741, or the office of the Institutional Review Board at 305-5883 so that I can review the matter and identify the medical resources which may be available to me.

I understand that:

a) The Presbyterian Hospital will furnish the emergency medical care determined to be necessary by the medical staff of the hospital;

b) I will be responsible for the cost of such care, either personally or through my medical insurance or other form of medical coverage;

c) No monetary compensation for wages lost as a result of injury will be paid to me by the Columbia Presbyterian Medical Center.

d) I will receive a copy of this signed consent form.

Signed_____

Date_____

Print
Name_____

Witnessed_____ Date_____

The Institutional Review Board of the Columbia Presbyterian Medical Center has approved the recruitment of subjects for this study.

CONSENT FORM
The Multicenter Study of Diet and Lipoproteins in Humans

You are invited to participate in a research study being conducted by the University of Minnesota and the Clinical Research Center. You were selected as a possible participant because you are healthy, between the ages of 22 and 72 and have a blood cholesterol level between the 25th and 90th percentile. Please read this form carefully and ask any questions you may have before agreeing to take part in the study.

Background Information:

The purpose of this study is to determine how diets containing different kinds and amounts of fatty acids affect blood lipids, hemostatic factors, antioxidants, insulin and glucose in free-living, healthy subjects. The information gained from this study will help us develop better nutritional guidelines for preventing heart disease.

Study Procedures:

This study is examining how different types of diets which we provide for you affect cholesterol and other blood fats which can influence your risk for developing heart disease. The information gained from this study will help us develop better nutrition guidelines for preventing heart disease. The study will include three eight week diet periods and you will eat three different diets during this time. (All of the diets will consist of standard, commonly used foods. No experimental food components will be included.) Menus of the various meals are available for your review.

You will be on three different diets containing a different amount of fat during each of the three diet periods. The fats used will include olive oil, corn oil, safflower oil and butter. The foods within each diet period will be rotated on a 8-day basis. The meals will be designed to fit your nutritional needs and will maintain our current body weight. You will be required to come to eat breakfast and dinner at the study facility Monday through Friday. You will pick up prepackaged containers of lunch and a snack for your use at home or work. Weekend meals also will be prepackaged for your convenience.

You are required to eat ONLY the food provided by the Feeding Center. If you use alcohol, you will be required to limit your intake to 5 drinks per week. Caffeinated beverages will be limited to no more that 5 in a day. Your body weight will be taken daily to allow calorie adjustment to maintain your current weight. Approximately two tablespoons of blood will be drawn four times during each diet period. You will be asked to complete two 24-hour urine collections per eight week diet periods.

Following each diet period, you will be given a break when you can eat any food you want and during which you do not need to come to the study facility.

Risks and Benefits of the Study:

The study has minimal risks. The blood drawing may cause bruising or fainting. Infection is a remote possibility. Every effort will be made to minimize these risks. The study diets will be designed to meet your daily nutrient needs.

A benefit of participation is that you will receive free, nutritious food. You will also receive the results from the blood analyses. You will receive $150 after completing each of the first two 8-week diet periods and an additional $500 after completing the third and last diet period.

Compensation:

In the event that this research activity results in an injury, treatment will be available, including first aid, emergency treatment and follow-up care as needed. Payment for any such treatment must be provided by you or your third party payor, if any (such as health insurance, Medicare, etc.)

Confidentiality:

The records of this study will be kept private. All data will be kept in locked files and identifiable by ID number. Any reports we publish will not include information that will make it possible to identify you as a subject.

Voluntary Nature of the Study:

Your decision whether or not to participate will not affect your current or future relations with the University of Minnesota or the Clinical Research Center. If you decide to participate, you are free to withdraw at any time without affecting those relationships. If you choose to withdraw from the study, you will be paid only for the diet periods you have fully completed.

Contacts and Questions:

The researchers conducting this study are Drs. Patricia Elmer, Joanne Slavin and Aaron Folsom. You may ask any questions you have now. If you have questions later, you may contact them at (612) 624-1818.

You will be given a copy of this form to keep for your records.

Statement of Consent:

I have read the above information. I have asked questions and have received answers. I consent to participant in the study.

Signature_____ Date_____

Signature of Study Coordinator_____ Date_____

Informed Consent

DIETARY EFFECTS ON LIPOPROTEINS
AND
THROMBOGENIC ACTIVITY
(DELTA)

I _____, voluntarily consent to participate in the research study entitled The Dietary Effects on Lipoprotein and Thrombogenic Activity. This research program is the first multi-center controlled feeding study on the effects of diet on coronary heart disease risk factors. The Pennington Biomedical Research Center is one of four centers in the United States participating in this controlled feeding study.

Heart disease remains the leading cause of death in Americans and elevated blood cholesterol is a primary risk factor for this disease. The development of blockages in blood vessel along with abnormal blood clotting can effect heart functioning. It is important to determine what diet changes have a beneficial effect on blood cholesterol and blood clotting in order to better understand how to control and prevent this disease. Controlled feeding studies are needed to scientifically determine what dietary changes may be most beneficial.

I understand that by volunteering to participate in this study I am agreeing to consume a diet provided by the Pennington Biomedical Research Center. All foods and beverages will be provided by the center. I agree not to consume any foods or beverages from outside sources with the exception of dinner on Saturday which will be considered a free choice meal. The diets will consist of wholesome foods that meet the recommended dietary allowances for essential nutrients and will be adequate in calories in order to maintain my current weight. I understand that I will be fed three separate diets throughout the course of the study. A different diet will be tested during each of the three eight - week diet trials. I agree to participate in interviews and questionnaires in order to assess my compliance to the diets.

I understand that I will be weighed twice a week before breakfast to monitor the adequacy of the diets. I agree to eat a minimum of ten meals per week at the center. I understand that strict adherence to the diet schedule is required and that this may interfere with social activities such as dining in restaurants. Even though menus are carefully planned to include as much variety as possible I am aware that the food selection will be restricted compared to what is available in the supermarket. This limited selection may result in some boredom with the diet over the course of the study.

I am willing to have a blood sample taken from my arm once per week during the last four weeks of each eight - week diet period. This means that I agree to have my blood sampled 12 times during the 24-week study. The amount of blood taken each time will be limited to two tablespoons. Possible risks associated with obtaining blood samples include discomfort as the needle is inserted into the vein in the arm. There may be some local bruising or swelling at the point of entry of the needle and in rare cases a blood clot or bleeding from the site may occur.

Foods will be prepared according to accepted standards of sanitation and provisions will be made to ensure the safety of foods provided for off site consumption. However it is possible that contamination during shipping, storage or preparation could go undetected and result in food born illness. Every effort will be made to guard against this occurring. I understand that upon receipt of the packaged meals I am responsible to refrigerate them to protect against food born illness.

I understand that all meals, procedures and assessments will be provided at no expense to me. I will be paid a stipend totaling $900 after completing all phases of the study. I will be paid in increments of: $100 after the first eight weeks; $300 after the second eight weeks; and $500 upon completion of the study. I understand that my participation may be terminated if I am found not to be compliant with study requirements. I understand I will be compensated financially only for the phases of the study I have successfully completed.

I understand that through my participation in this study I will be contributing to the body of knowledge in biomedical science. I have been informed that the results of the study may be published but that my privacy will be protected and my name will not be published.

I am aware that monitors from the National Heart, Lung and Blood Institute or members of the Committee on Human Research may need to see my study results in order to verify data. In the event that this occurs I understand that my privacy will be protected.

The study has been explained to me in detail and I have been given an opportunity to ask questions of Dr. Michael Lefevre and the staff of Clinical Trials. I understand the nature and purpose of the study and of the various tests and their potential risks. I understand that I may contact Dr. Lefevre at (504) 765-2569. I may contact the staff of Clinical Trials at (504) 765-2578 for questions at any time during the course of the study.

I have thoroughly read the above and understand what is involved in participating in this controlled diet study. My participation is entirely voluntary and I am free to withdraw from the study at any point without repercussions.

_____ _____

Signature of Participant Witness

_____

Date

_____

Investigator

_____

Dr. George Bray M.D. or Dr. Donna Ryan M.D.

DELTA PROTOCOL 1

CONSENT TO PARTICIPATE
IN THE
Diet Effects of Lipoproteins and Thrombogenic Activity Study
(DELTA)

Penny M. Kris-Etherton, Principal Investigator
Nutrition Department
The Pennsylvania State University
University Park, PA 16802

I agree to participate as a research participant in the Diet Effects on Lipoprotein and Thrombogenic Activity Study (DELTA). This research program is the first multicenter controlled feeding study of dietary effects on coronary heart disease risk. Four centers in the U.S. will enroll a total of 100 participants for this study, who will be given a controlled feeding regimen that is identical in all centers.

Propose of the study

Heart disease is the leading cause of illness and death in Americans, affecting millions of men and women. Coronary heart disease (CHD) affects the heart muscle, mainly as a result of atherosclerosis and its complications. Atherosclerotic plaques and thrombosis combine to interrupt blood flow to the heart, producing the clinical systems of the heart disease and heart attack. Elevated blood cholesterol (fat in the blood), is a primary risk factor for heart disease. Abnormalities in blood clotting factors contribute to thrombosis. Dietary measures that have a beneficial effect on blood cholesterol and clotting could reduce the burden of CHD in Americans. In order to determine how dietary modifications will benefit the general public, large numbers of participants need to be studied under very well controlled conditions.

Procedures

I understand that my participation in DELTA will involve the following procedures:

1. At the beginning of the study, I will be asked questions about my medical history, current medications, and questions about my dietary habits, dietary restrictions, and level of physical activity. I will also be asked to provide a fingerstick blood sample during phase 2 of screening and a 1.25 oz. blood sample during phase 3 of screening to determine blood cholesterol, blood chemistry, and hematology.

2. I will be asked to follow a diet in which all foods and beverages are provided. I will be asked to eat everything provided and not to eat any food or beverages from outside sources (except for one self-selected meal on a weekend evening). The diets will consist of wholesome foods that meet the Recommended Dietary Allowances (RDA) for essential nutrients and adequate calories to maintain my present weight. The experimental diets will be modified in type and amount of fat.

DELTA PROTOCOL 1

3. I will be asked to fill in a daily food record check list when I come to the feeding site. I will be asked to eat a minimum of 10 meals per week at the feeding center, 5 lunches and 5 dinners and will be provided take-out meals for the remaining meals and snacks. I will have my weight measured two times every week.

4. I will be asked to provide adequate refrigeration to store foods that are provided for offsite consumption.

5. I will also have blood samples drawn from a vein in my arm at regular intervals during the study, for the measurement of total cholesterol, HDL, and LDL cholesterol, blood clotting factors and for a variety of blood tests which will evaluate my overall state of health. Each blood sample will involve approximately 2 ounces of blood. A total of 12 samples will be collected at regular intervals during the study. Four will be taken during each of the three eight week diet periods. One blood sample will be taken on the following weeks of each diet period: Week 5, Week 6, Week 7, Week 8.

I understand that all food will be provided in the Mateer Room in Henderson Building (North). Onsite meals will be served between 11:30 and 1:30 (lunch), 4:30 and 6:30 (dinner). Filling in the daily food record will take about 5 minutes.

Risk/Discomforts

The diets that will be fed will consist of wholesome foods and contain adequate levels of essential nutrients. Foods will be prepared according to accepted standards of sanitation and provision made to ensure the safety of foods provided for offsite consumption. However, it is possible that incorrect food handling during shipping, storage, or preparation if not detected could result in food-borne illness. Every effort will be made to safeguard against this possibility.

Feeding studies that require onsite eating of meals and strict adherence to the diets provided may interfere with social activities centered around eating such as dining in restaurants. While rotating menus will provide some variety in the diets, the number of food items will be more limited than available in an average grocery store. The limited variety may become boring over the course of the study.

Other risks of the study involve those of taking blood. These include: commonly, the occurrence of discomfort and/or bruising at the site of the puncture: and less commonly, the formation of a small blood clot or swelling of the vein and surrounding tissue and/or bleeding from the puncture site.

Possible Benefits

Elevations in cholesterol values may be associated with a greater risk of developing atherosclerosis (fatty deposits in the arteries) and coronary heart disease such as angina or heart attack. The benefits of participation in this study include the possibility that I will experience lowering of my blood cholesterol, improvement in some clotting factors, and that I may enhance scientific knowledge concerning the most effective diet modifications to lower the risk of CHD. No benefit from this treatment is guaranteed.

DELTA PROTOCOL 1

Alternative Therapies

This is not applicable because there are no alternative therapies. This is a diet study, not a drug study.

Costs

There will be no cost to me for my participation in the study. I will receive all food free of charge during the study.

Participation and Termination

Participation in this study is entirely voluntary. Participants are completely free to withdraw from the study at any time.

I will be informed of any new information that may affect my willingness to participate. Any questions that I may have concerning any aspect of this study will be answered by Dr. Kris-Etherton and by other members of the clinical staff. I also understand that I am free to refuse to participate or to withdraw from participation in this study at any time.

Dr. Kris-Etherton should be notified immediately of any new condition or injury which develops during the course of the study. The Pennsylvania State University, which is sponsoring the research, does not furnish any funding for medical treatment or compensation for human subjects in the event the research results in physical injury. In the event of such injury, the University will make available only immediate and essential medical treatment including hospitalization. For further information about this I may call the Office of Regulatory Compliance at 865-1775.

I understand that I may be asked to leave the study at any time if I do not comply, and if I am discontinued, I will be compensated only for the amount of time spent at the time my participation was terminated.

Confidentiality

My research records will be handled as confidentially as is possible within the law. All records are coded with an I.D. number and no names are transmitted to the central data processing center. Records containing names or other identifying information are kept under lock at the clinical center, and only study investigators have access to them. Under certain circumstances, monitors from the National Heart, Lung and Blood Institute or members of the Pennsylvania State University Committee on Human Research may need to see some specific records in order to verify the study data. No individual identities will be used in any reports or publications resulting from this study. At the end of the study, I will be given my laboratory results without cost, informed of the results of the study, and advised on their implications for my future care.

DELTA PROTOCOL 1

Rights of Investigators

- Right not to enroll a subject

- Right to terminate for the following reasons:
  - participant noncompliance
  - very poor attitude of a participant that is disruptive to the study.

I have read and understood the consent form and have had an opportunity to discuss this study with a member of the clinic staff. All my questions regarding my rights as a research participant concerning this study have been answered to my satisfaction and I hereby willingly consent to participate in this study. A copy of this consent form has been given to me.

Signature of Participant	Signature of Witness
Date	Date

Signature of Investigator

Date

APPENDIX B

COMMUNICATIONS

Communications

Design characteristics of single-center versus multicenter trials differ in the fact that single-center trials are easier to design and operationalize because all study personnel are located in the same institution. For single-center trials, it is not necessary to maintain communications and decision-making structures for execution of the trial. In addition, the physical proximity of study personnel may make it possible for them to work more efficiently and to achieve a higher degree of uniformity in the procedures they perform than might be expected in a multicenter trial. The main weaknesses of a single-center trial are the sample size and resource limitations. One center and a few investigators will find it difficult to recruit and follow the numbers of patients needed (Meinert, Curtis L, CLINICAL TRIALS: Design, Conduct and Analysis, Oxford University Press, New York/Oxford 1986).

The communications network of a multicenter trial relies on frequent and timely communiques during the design, implementation and conduct of a research trial. This makes it imperative that the decision-making structures, the Steering Committee and all the subcommittees, provide the facility to communicate in such a fashion that all timeframes and deadlines are met with some reasonable degree of timeliness. The vast differences in individual's daily work schedules and traveling schedules makes the burden of interactive communications a serious one.

Purpose

To provide a structure to facilitate timely, efficient, and economical transmission of information between the DELTA Coordinating Center, central laboratories, field centers, and NHLBI program office.

Communications from the Coordinating Center

The Coordinating Center is responsible for ensuring that the DELTA protocols are properly implemented and carried out and for protecting the integrity and security of the data. This responsibility is carried out through assisting in the development and distribution of protocols, procedure manuals, forms, and data entry systems. The Coordinating Center maintains a continuing quality assurance program through ongoing monitoring of data, site visits, inspection of forms completion, and assay of the experimental diets. Any problems encountered in this process will be reported to the appropriate principal investigator, sub-committee Chairperson, NHLBI project scientist, or Steering Committee.

Communications from Field Centers

Field Centers and central laboratories have a responsibility for on site monitoring of the protocol and procedures. It is important that any ambiguities in the written procedures be brought to the immediate attention of the Coordinating Center. Likewise, deviations from protocol or problems encountered in carrying out the protocol should also be brought to the immediate attention of the Coordinating Center.

Procedures

eMAIL

Each center and central laboratory has the facility to send and receive electronic mail either through the equipment supplied by DELTA or through their own email systems. eMAIL will be the main conduit

for transmitting information and documents originating from the Coordinating Center. Each facility is responsible for providing the correct eMAIL addresses to Ms. Pat Robinson at the Coordinating Center (telephone #: 919-962-3223, eMAIL address: UCCPLR.CSCC@MHS.UNC.EDU) and ensuring that the person receiving the eMAIL transmits it to the addressee.

Federal Express mail

Use of Federal Express will be limited to bulky items requiring a quick turnaround.

Each field center and central laboratory should designate a staff person who will receive all Federal Express mail and distribute copies within the center. The Coordinating Center will maintain a log and confirmation of all Federal Express mail sent and received. Questions concerning Federal Express shipments should be directed to the DELTA secretary, Ms. Pat Robinson at 919-962-3223.

FAX

The Coordinating Center will continue to fax short documents that cannot be distributed through eMAIL. Each facility is responsible for ensuring that fax boxes are checked on a regular basis. Problems receiving faxed material should be directed to Ms. Robinson.

Telephone

Conference calls can be a very costly way of transmitting information and should be limited to resolution of issues that require discussion. In order to make conference calls most efficient, agendas, issue papers and background documents should be sent in advance, with a clear designation of actions that need to be taken in the conference call. Conference calls should not be used as a forum for providing progress reports. This is done more efficiently and cheaply through eMAIL.

The Coordinating Center will set up all conference calls through the local DAIN operator. Approximately 15 minutes before the scheduled time for the conference call, the DAIN operator will begin calling each person. It is important that each conferee either be available to answer the telephone or have someone else available to answer the phone 15 minutes before the scheduled call. Otherwise, the DAIN operator will have to redial the number and this wastes everybody's time. If you have problems getting connected, or you wish to add someone to a call, contact Ms. Robinson immediately and she will contact the DAIN operator.

Individual telephone calls and messages

E-mail is a very efficient way of carrying on a dialogue with colleagues since it avoids the hassle of playing telephone tag and provides a permanent record of the communication. It only requires the recipient to open the eMAIL or designate someone to check the eMAIL on a regular basis.

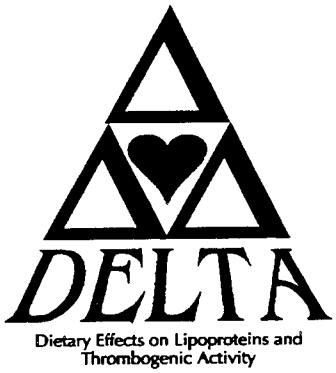
The Coordinating Center switchboard is open Monday through Friday from 8:00 am until 5:00 P.M.

The DELTA research staff is available to answer your questions during that period.

For questions related to laboratory issues, data entry, data management and data analysis, call Ms. Nancy Anderson (telephone #: 919-962-3052, eMAIL address: UCCNYA.CSCC@MHS.UNC.EDU). Alternate, Susan Blackwell (telephone #: 919-962-3092, eMAIL address: UCCSEB.CSCC@MHS.UNC.EDU).

For questions related to the Steering Committee, DELTA-2 forms and procedures, call Susan Blackwell (alternate, Nancy Anderson)

For questions relating to the sub-committees, publications, food procurement, call Lynn Martin (telephone #: 919-962-3096, eMAIL address: UCCLMM.CSCC@MHS.UNC.EDU). (Alternate Susan Blackwell).



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