

Chapter 18

PROCEDURES FOR CENTRAL LABORATORY SPECIMEN COLLECTING, SHIPPING AND PROCESSING

18.1 INTRODUCTION

Selected AREDS Clinical Centers will be participating in the study of serum levels of vitamins A, E, C, and beta-carotene, zinc, copper and cholesterol. The procedures outlined below apply to these centers. For a long-term study such as the Age-Related Eye Disease Study (AREDS), it will be very important to assure that continuity of all techniques is maintained throughout the study duration, to minimize variability by clinic or by technician. This chapter is designed to provide all necessary information for correct collection, processing, and shipment of the AREDS blood specimens for analysis, as well as a summary of procedures used by the Central Laboratory for processing the blood once received. An overview of the collection, processing and shipment procedures is shown in Exhibit 18-1.

Fasting blood samples (after at least a 4-hour fast) will be collected by each participating Clinical Center from each participant in the AREDS. Hematocrit determinations will be performed locally in all Clinical Centers (Section 7.9).

18.2 CENTERS FOR DISEASE CONTROL (CDC)

The Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, serves as the AREDS Central Laboratory for Nutritional and Blood Biochemistry. Blood samples for all AREDS participants at participating Clinical Centers will be sent to the Central Laboratory for determination of levels of vitamins A, C, E, carotenes, zinc and copper. In addition, laboratory determinations of fasting levels of total cholesterol, HDL cholesterol, and triglycerides will be performed.

18.3 TRAINING AND CERTIFICATION OF CLINICAL CENTER STAFF

All Clinical Center staff participating in Central Laboratory studies will be thoroughly trained in each aspect of the specimen collection, processing, and shipping procedures. Training will take place at an initial training session held at the Central Laboratory. Clinical Centers participating in the Central Laboratory blood studies are encouraged to have at least two AREDS staff members trained and certified in these procedures. It is expected that this will help to minimize any inconvenience to participants should a staff member certified in the Central Laboratory procedures be absent from the clinic. Training of personnel not attending this training session will take place at the Clinical Center by a staff member trained at the Central Laboratory training session and (at least) provisionally certified in conjunction with staff from the Central Laboratory. Members of the Training and Certification Faculty who attended the Central Laboratory training session may assist in the training and certification process.

Following completion of training, clinic personnel applying for provisional certification must take blood samples from two persons who are not AREDS participants. The samples should then be prepared according to the procedures outlined in this chapter and shipped to the Central Laboratory. When the shipment is received at the Central Laboratory, the laboratory supervisor will review the documentation and evaluate the specimens. If the samples and documentation meet the protocol requirements, provisional certification will be granted to the candidate and the Protocol Monitor at the Coordinating Center notified. The Protocol Monitor will notify the candidate when provisional certification is obtained. Clinic personnel who have achieved provisional certification will be granted full certification by an AREDS certifier after they satisfactorily complete a performance test observed by the certifier.

18.4 SPECIMEN COLLECTING AND PROCESSING

In four of the Clinical Centers, blood specimens will be obtained at the Qualifying Visit from fasting participants (after at least a 4-hour fast) who have not smoked or been exposed to cold or exertion for at least half an hour prior to specimen collection. If it is not possible to obtain the sample at the Qualifying Visit, the sample may be taken at the Randomization Visit. Three of the Clinical Centers will obtain blood specimens at each Annual Visit. If the Annual Visit is missed, the sample may be obtained at the next Nonannual Visit. Specimens will be prepared for determinations to be performed by the Central Laboratory. Equipment that should be available at a participating center and should be assembled beforehand includes:

- (1) blood-collection chair
- (2) a centrifuge capable of 1500 X G
- (3) freezer (not frost-free, preferably at least -20 °C, with a high-temperature alarm)
- (4) latex or vinyl disposable gloves (powder-free)
- (5) 20- or 21-gauge multisample Vacutainer needles and holders
- (6) gauze, alcohol swabs, bandages, and tourniquets
- (7) a vortex mixer
- (8) test tube racks
- (9) permanent markers
- (10) labels (provided by the Coordinating Center)
- (11) Pasteur pipettes
- (12) disposable paper towels
- (13) biohazard disposal containers.

In addition to the equipment listed above the following materials supplied by the Central Laboratory should be assembled:

- (1) 7-mL royal blue-top trace metal Vacutainers
- (2) white- or red-filter 13-mm serum separators, in resealable bags
- (3) 2-mL high-density polypropylene storage vials (including a set in resealable bags, to be used for zinc/copper specimen collection only)
- (4) 15-mL red-top SST Vacutainers
- (5) 250- μ L and 1.0-mL micropipettors and tips for vitamin C

- (6) pre-weighed vials containing 3 g metaphosphoric acid, for vitamin C specimen preparation

Do not use the Central Laboratory-supplied materials for collecting or processing blood for any other study tests; they have been prescreened for background trace element contamination and it is imperative to keep them as clean as possible.

Other miscellaneous phlebotomy supplies should be purchased and maintained by participating centers.

18.5 PREPARATION AND BLOOD COLLECTION

18.5.1 Summary

Collect and completely fill, in this order, the following Vacutainers from each participant:

- (1) **1 15-mL red-top** (plain)
- (2) 1 Purple top EDTA tube (hematocrit); size specified by local laboratory requirements. (Hematocrit to be processed locally. Do not send to the Central Laboratory. See Section 7.9).
- (3) **1 7-mL royal blue-top** (trace metal)

18.5.2 Preparation

Assemble blood collection materials such as needles, gauze pads, alcohol swabs, bandages, Vacutainers and holders, tourniquets, labels, vials, and needle disposal containers in advance. Wear disposable latex or vinyl gloves.

Before the blood specimen is drawn, have the participant rest in a seated position for at least 5 minutes and remain in this position during the venipuncture. (Postural variation may elevate the lipid results by as much as 10 to 15 percent.) The participant should have been fasting for at least 4 hours and should not have smoked, been exposed to cold, or exerted himself or herself for at least half an hour prior to the specimen collection. Clothing should not restrict the arm.

18.5.3 Blood Collection

Draw the blood from an antecubital vein or from some other convenient arm vein. Apply the tourniquet to locate the vein, then release it while the venipuncture site is being cleaned. Swab the venipuncture site with alcohol and allow it to dry. Reapply the tourniquet, reconfirm the vein location, and perform the venipuncture. Care should be taken to avoid protracted probing for vein location. Release the tourniquet before the needle is removed from vein. Prolonged application of the tourniquet should be avoided to minimize hemoconcentration.

Collect blood using a Vacutainer system following the instructions supplied with the tubes. (Training tapes are available from the manufacturer, Becton-Dickinson, Rutherford, NJ.) Introduce the needle into the vein and fill the Vacutainers as completely as possible, collecting the red-top tube first, the purple-top tube second, and the blue-top tube last.

Use a dry gauze pad to apply pressure when removing the needle since a wet pad could result in fluid being drawn into the Vacutainer. After removing the needle, have the participant apply pressure firmly over the puncture site with his arm held straight and elevated over the head for several minutes, rather than with the elbow bent, to avoid hematoma formation. Re-examine the puncture site to verify that any residual bleeding has ceased after five minutes, then apply a bandage as a precaution. Dispose of all needles and contaminated wastes properly in the biosafety container.

18.5.4 Safety Note

The Central Laboratory recommends as good laboratory practice that all blood specimens, used needles, and other items associated with specimen collection should be treated as though they were infectious for HIV and hepatitis B virus. All used needles should be placed in puncture-resistant containers; then, along with used gauze, Vacutainers, pipets, vials, etc., they should be autoclaved prior to disposal. Use of disposable gloves when collecting and processing blood is also required. (See Centers for Disease Control & Prevention. Recommendations for prevention of HIV transmission in health-care settings MMWR 1987;36 (suppl 2S) and Centers for Disease Control & Prevention. Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HBV, HCV, and HIV and Recommendations for Postexposure Prophylaxis MMWR 2001;50 (RR11):1-42).

18.6 EXTRACTING SERUM

After the blood specimens have been drawn, allow the filled Vacutainers to remain at room temperature for 30-45 minutes but no more than one hour for complete blood coagulation. Keep tubes away from sunlight or any other strong light source, which may degrade the vitamin A content of the serum. If exposure to a room window is unavoidable, then tubes should be temporarily wrapped in aluminum foil for protection. If necessary, the blood specimens may be allowed to coagulate for 30 minutes and then refrigerated for up to 2 hours to allow more time between the collecting and processing phases. This is not recommended as a part of routine practice but for use in special circumstances.

Centrifuge the red- and blue-top tubes for 15 minutes at 1500 X G (or approximately 2400-3000 RPM for most counter-top centrifuges). Do not "rim" tubes prior to centrifugation; this may introduce contamination. Be sure to use balance tubes in the centrifuge if necessary. The time required for centrifugation is a convenient time to prepare the remaining supplies for specimen processing, to create a specimen list and to organize the vials and labels.

18.7 PREPARING SPECIMENS

18.7.1 Summary

When completed, serum preparation will result in 5 vials as follows:

- (1) VIAL 1 ("CHOL") -- serum for cholesterol/triglyceride;
- (2) VIAL 2 ("HDLC") -- serum for HDL cholesterol;
- (3) VIAL 3 ("ZINC") -- serum for zinc/copper;
- (4) VIAL 4 ("VITA") -- serum for vitamin A/E/carotenes;
- (5) VIAL 5 ("VITC") -- serum for vitamin C;
- (6) VIAL 6 ("RESV") -- excess serum in a reserved or archived vial.

18.7.2 Red-top Tube Processing

Plan the participant schedules so that all steps required for preparing the vials for shipment can be performed within the acceptable time limits. Aliquot the serum specimens into 5 labeled 2 mL high-density polypropylene vials for shipment as described below. Use clean glass Pasteur pipets or disposable one-piece plastic transfer pipets for removing serum from the red-top tubes only.

Prepare 5 specimen vials using the serum from the red-top tube for shipment to the Central Laboratory:

VIAL 5 Preparation: Once a week, prepare a working solution of 6 g/dL metaphosphoric acid (MPA) for the vitamin C specimen. This is done by opening a pre-weighed 3 g/vial MPA (supplied by the Central Laboratory) and diluting to 50 mL with distilled or de-ionized water. Mix well. It will take about 15-30 minutes for all of the MPA to go into solution, so this should be done as the first step each week. **NOTE:** MPA acid is very hygroscopic; it takes up water and decomposes to form phosphoric acid. It also decomposes to phosphoric acid in solution gradually. Therefore, it is very important to handle the MPA solution carefully as a strong acid, and to properly discard the unused solution each week it is prepared. **Do not try to save it beyond one week. Prepare a fresh solution each week that it is needed. Refrigerate MPA solution between each day when not in use.** Adequate MPA vials will be sent to supply each participating clinic for every year of the study.

The following pipetting steps must be done accurately. Using the 250- μ L MLA micropipettor and a new tip, place 250 μ L of participant serum in a 6 mL polypropylene vial labeled "VITC." Add 1.0 mL of the 6 g/dL MPA solution with the 1.0 mL MLA micropipettor, then cap the vial tightly and mix contents well using the vortex mixer. This dilution step (1 part serum plus 4 parts MPA) stabilizes the vitamin C. This serum-MPA mixture may be stored at -20 to -70 degrees C for up to 6 months.

Use a fresh tip for each participant's serum; the same larger size tip may be used all day for the MPA solution. Do not use these pipettors for any other assays or studies, so that they will maintain calibration. (**NOTE:** It will be necessary to periodically check calibration of the pipettor

and keep a log of the results. If a pipet appears to be definitely out of calibration, notify the Central Laboratory for a replacement while this pipet is repaired.)

VIALS 1, 2, 4, and 6 Preparation: Using a clean Pasteur pipet, and taking care not to introduce red blood cells from the clot, place the remaining serum from the red-top tube in vials 1, 2, 3, and 6 in the following volumes:

- (a) Vial 1 - **1.5 mL**, vial labeled "**CHOL**"
- (b) Vial 2 - **1.5 mL**, vial labeled "**HDLC**"
- (c) Vial 4 - **1.0 mL**, vial labeled "**VITA**"
- (d) Vial 6 - all of residual serum, vial labeled "**RESV**"

**** NOTE:** Any additional serum beyond the capacity of vial 6 to the 2.0-mL mark may be equally divided among the other vials.

Close all vial caps tightly to prevent leakage. Make sure labels are applied neatly going along the length of the vial with the left end of the label pointing toward the top of the vial and the right end towards the bottom. Place vials in a 9 x 9 grid box with each patient's samples in a single row. Each column should contain samples for a particular analysis (zinc/copper, vitamin C, etc.).

Keep the box of Pasteur pipets covered when not in use to avoid contamination. Dispose of the Vacutainer tube and Pasteur pipet properly.

18.7.3 Royal Blue-top Tube Processing

Prepare one specimen vial using the serum from the royal blue-top tube for shipment to the Central Laboratory. For processing the trace metals tube, the Central Laboratory recommends the use of powder-free gloves. If regular powdered gloves are used, the risk of contamination can be reduced by rinsing your hands under distilled or deionized water immediately after putting on the gloves. Be sure to dry your hands carefully to avoid tearing the gloves, before you begin processing the royal blue-top tube.

VIAL 3 Preparation: Analysis of serum specimens for zinc/copper levels requires special specimen preparation techniques. The Central Laboratory will supply 7 mL royal blue-top trace metal Vacutainers, white- or red-tipped serum separators, and precapped vials, all in resealable bags, to be used for zinc/copper specimen collection only. Do not use these materials for collecting or processing blood for any other study tests; they have been prescreened for background zinc/copper contamination and it is imperative to keep them as clean as possible.

Allow the blood in the trace metals Vacutainer to clot as previously described. Do not open the Vacutainer before centrifugation; do not use a wooden application stick to rim the clot; do not use the uncapped vials; and do not use a glass or plastic pipette to remove the serum. Use only the white- or red-filter serum separator for this procedure.

The zinc/copper serum specimen will be put in one of the precapped polypropylene vials that were packaged separately in zip-lock bags. Do not remove these vials or the serum separators from

their zip-lock bag until you are ready to process the centrifuged serum. Reclose the bag immediately thereafter to keep these supplies as clean as possible.

The ideal conditions for processing this specimen would require a laminar-flow hood to work under when separating and aliquotting the serum; however, since that is not possible in the clinics, the Central Laboratory has tried to prepare these materials so that they will have a minimum of contamination from background zinc/copper present in the processing area.

After centrifugation, remove the stopper from the Vacutainer. Carefully and slowly push the serum separator tube into the Vacutainer, white filter end down. Do not force it quickly: this could possibly create aerosol formation of the serum. The end of the filter should stop just short of the clot/serum interface. The entire serum yield may now be decanted into the vial labeled "ZINC." Recap the vial immediately after filling it with serum. Red blood cells should not be forced into the serum; they contain very high amounts of zinc compared to the serum levels and a falsely elevated serum zinc value would result. Also, if the serum in this tube is grossly hemolyzed, do not submit it for analysis. Return the labeled but empty vial and note "hemolyzed" on the label.

18.7.4 Specimen Freezing and Vacutainer Disposal

Place vials 1-6 in a 9 x 9 grid box for shipment to the Central Laboratory, and secure the box with a zip-lock bag. Freeze the specimens upright so that serum does not collect in the vial cap at -20 degrees C or lower until shipment time.

When discarding the trace metals Vacutainer, do not remove the separator. Discard them together as one unit into the autoclave bag for biological materials to minimize any hazard from potentially infectious specimens.

18.8 SHIPPING SPECIMENS

Specimen shippers will be supplied by the Coordinating Center; Federal Express labels will be supplied by each Clinical Center. Use either cube or "pellet" dry ice (12 lbs per shipper). Fill out the standardized shipping list supplied by the Coordinating Center itemizing participant identification numbers, name codes, numbers of vials collected, and dates of collection and mailing for each shipment. By each participant's ID, neatly place a bar-coded label for ID verification upon receipt. Add any pertinent comments regarding condition of specimens such as "inadequate blood draw," "all specimens hemolyzed," "all specimens grossly lipemic," "specimens allowed to thaw inadvertently," "specimens refrigerated prior to processing," etc. Include one copy of the shipping list with the shipper under the outer lid, and fax a copy to the AREDS Central Laboratory at the CDC (404-488-4609) to track shipments. Retain a third copy in the clinic for your records.

When packing the shipment, follow these instructions:

- (1) Put a layer of dry ice on the bottom of the shipper.
- (2) Cover with several paper towels or sheets of newspaper.
- (3) Place the boxes of specimens on the paper towels.

- (4) Cover with another layer of towels.
- (5) Add the rest of the dry ice (Do not use styrofoam chips).
- (6) Make sure the styrofoam inner lid is completely closed to preserve dry ice life.
- (7) Place the shipping list on top of the styrofoam lid, then secure the outer cardboard lid firmly with clear or nylon reinforced strapping tape, and attach the Federal Express label. Excessive amounts of tape are not necessary, since the shippers will be returned to each clinic every month.
- (8) Place a filled-in copy of the label in Exhibit 18-2 (which may be copied and cut to size) on the side of the shipper.

Send all shipments to the Central Laboratory by Federal Express using the schedule provided by the Central Lab. Ship no later than Wednesday in a week so that the shippers will be received at the Central Laboratory and the sample integrity preserved. Do not mail to arrive on a holiday; shipment one week later will be acceptable in this case only.

Address shippers as follows:

Dan Huff - Nutrition Lab
Bldg. 17 Loading Dock
Centers for Disease Control
4770 Buford Highway NE
Atlanta, GA 30341-3724
770-488-7932

Include your clinic address and telephone number on the label. If you have any questions, you may contact Dan Huff, MT (ASCP) (770-488-7932) or Rosemary Schleicher, PhD (770-488-4424).

18.9 CENTRAL LABORATORY PROCEDURES

18.9.1 Shipper Receipt at the Central Laboratory

Once a shipper arrives at the Central Laboratory, it will be logged in by the receiving clerk. Specimens will be removed and placed at -20 °C for short-term storage (several days at most). Dry ice will be removed from the shipper, which will be allowed to dry. Excess labels will be removed carefully, the shipper will be resealed, and returned by surface mail to the originating clinic for reuse. Periodically, new outer cartons will be provided for the styrofoam inner shells.

Since all shipments should arrive at the Central Laboratory the same week of the month, the clerk will hold all specimens for racking at one time. Shipments will be sorted by clinic, and verified against the shipping lists enclosed with the shipper. Any problems such as broken or leaking vials will be brought to the attention of the laboratory supervisor.

Specimens will be racked in sets of 20 for each analysis, with each set of 20 given a designated run name for data management purposes. The usual practice at the Central Laboratory is to designate the runs sequentially, according to a letter code indicating the month in which they

are received. For example, specimens received in the first rack of 20 in January (A) 1992 would be identified as A92A01; those in the fourth set in June (F) 1993 would be in rack A93F04, with the initial "A" indicating "AREDS." If analysis will not be performed within a short period of time, the specimens are placed in -70°C storage for maximum stability.

Vitamin levels will be determined using High Performance Liquid Chromatography (HPLC) with either UV/visible spectroscopy or electrochemical detection and mineral assays will be performed using Atomic Emission Spectroscopy. The lipid analyses will be performed on a Roche Hitachi 912 Analyzer. After all analyses are completed, the reserve serum aliquot without preservative may be held at -70°C at the Central Laboratory as an archival source, in case there are any questions about an analysis in the future, or if new technology emerges which may make additional analyses possible on these specimens to enhance the study database.

18.9.2 Data Transfer

The Central Laboratory will maintain a database of all blood level measurements obtained for each AREDS participant; the data will be indexed by Participant Registration Number. Data to be sent to the Coordinating Center will be stored in a Microsoft Access database. The results will be sent via e-mail at regular, monthly intervals in a comma-delimited text file (*.csv) attachment.

18.9.3 Reference Ranges for Serum Measurements

The reference ranges for serum measurements conducted by the Central Laboratory are shown in Exhibit 18-3.

18.9.4 Notification of Participants for Extreme Micronutrient Values

No participant or clinic will be informed of individual serum levels of micronutrients, except those participants found to have abnormal serum Vitamin A, as outlined in Section 18.10.

18.9.5 Certification

When new technicians are hired and trained for specimen collection/processing/shipping activities for the study, certification must be accomplished before that technician commences independent operation. After the new technician has been fully trained by study center staff, if possible, both the certified and new technician should prepare duplicate specimens and paperwork on non-enrolled persons for a shipment to be sent to the Central Laboratory. The Clinic Coordinator should indicate by memo that a new technician is participating in the study, and that his/her observed work was performed according to the manual guidelines. The Central Laboratory Coordinator will receive and review the shipment. If all vials have been properly prepared, and accompanying documentation is complete and correct, and the analyzed values are within the reference ranges, the Central Laboratory will notify the AREDS Coordinating Center, which will notify the technician with a statement of the acceptability of the work.

18.10 ABNORMAL SERUM VITAMIN A NOTIFICATION POLICY

1. In the event of the observation of serum vitamin A concentration ≥ 120 $\mu\text{g/dL}$ and ≤ 150 $\mu\text{g/dL}$ by the Central Laboratory, the Central Laboratory will analyze the specimen to determine the proportion of retinyl esters present. If less than 35 percent of the measured vitamin A content is present as retinyl esters, then no further follow-up is necessary, as such elevations have not, as yet, been associated with known pathological conditions.

The Central Laboratory will notify the appropriate Clinic Director of any of the findings listed below, with a suggestion for a confirmatory serum assay at the participant's next scheduled visit to the AREDS Clinical Center.

- ! Serum vitamin A concentration ≥ 120 $\mu\text{g/dL}$ and the proportion of serum vitamin A present as retinyl esters is at least 35 percent
- ! Serum vitamin A concentration > 150 $\mu\text{g/dL}$ regardless of proportion of retinyl esters present
- ! Serum vitamin A concentration < 20 $\mu\text{g/dL}$, regardless of proportion of retinyl esters present.

2. The Central Laboratory will notify the Clinic Director of the results of the analysis of the confirmatory specimen. If the result yields a serum vitamin A concentration of > 150 $\mu\text{g/dL}$ or < 20 $\mu\text{g/dL}$, or a serum level of > 120 $\mu\text{g/dL}$ with the proportion of retinyl esters present of at least 35 percent, then the Clinic Director will inform the participant. For high levels, the Clinic Director will inform the participant that the participant should discontinue taking Centrum®. For low levels, the Clinic Director will advise participants taking Centrum® to continue to do so. The Clinic Director will also inform the participant's personal physician of the results of the assays. The Central Laboratory will serve as a resource for the Clinic Director in determining the specific information to be communicated to the participant and the participant's personal physician.

18.11 GENETICS ANCILLARY STUDIES

A repository of genetic material from all AREDS patients will be completed by 2002. This resource can be made available to scientists to conduct research into the causes of cataract and macular degeneration. Development has occurred in two stages.

In Stage I, five AREDS clinics (Albany Medical Hospital, Devers Eye Institute, the National Eye Institute, Wilmer Ophthalmological Institute at Johns Hopkins Hospital, and the University of Wisconsin at Madison) sent blood to the Centers for Disease Control (CDC) in Atlanta, Georgia for processing. The first blood specimen from these five clinics was drawn in April 1998. Three tubes

of blood per patient were sent to the CDC for processing: one tube is used to extract DNA directly, one is used to make a permanent cell line, and one is used to isolate lymphocytes (white blood cells) and freeze them for quality control purposes.

In Stage II, the remaining six AREDS clinics (Associated Retinal Consultants, Emory University, Ingalls Memorial Hospital, Massachusetts Eye/Ear Infirmiry, University of Pittsburgh, and Elman Retina Group) sent blood to the Coriell Institute for Medical Research in Camden, New Jersey for processing. The first blood specimen from these six clinics was drawn in September 1999. Two tubes of blood per patient were sent to Coriell for processing: one tube of blood is used to extract DNA directly, and the other is used to make a permanent cell line.

Clinical instructions for specimen collection, processing, and shipping for Stage I and Stage II are contained in the Data Management Handbook. Processing center instructions for labeling the specimens are also available in the Data Management Handbook.

18.12 REFERENCES

1. Nutritional Biochemistry Branch, Centers for Disease Control and Prevention, Atlanta, Georgia. Unpublished data (see note 1 for Exhibit 18-3).
2. Gunter EW, Turner WE, Neese JW, Bayse DD. Laboratory procedures used by the Clinical Chemistry Division, Centers for Disease Control, for the Second Health and Nutrition Examination Survey (HANES II), 1976-1980. Atlanta: Centers for Disease Control, 1981. (Revised edition, 1985)
3. Fulwood R, Johnson CL, Bryner JD, Gunter EW, McGrath CR. Hematological and nutritional biochemistry reference data for persons 6 months - 74 years of age: United States 1976 - 1980. Vital and Health Statistics, Series II, No. 232. DHHS Publication No. (PHS) 83-1682, Washington, DC: U.S. Government Printing Office, 1982.

Exhibit 18-1. PROCEDURES FOR CENTRAL LABORATORY SPECIMEN COLLECTING, SHIPPING AND PROCESSING: OVERVIEW

1. MATERIALS PREPARATION (Section 18.4)

Supplied by Clinical Center

blood collection chair	bandages
centrifuge (1500 x G)	tourniquets
freezer	vortex mixer
powder-free latex/vinyl gloves	test tube racks
20- or 21-gauge multisample Vacutainer	permanent markers
needles/holders	Pasteur pipettes
gauze	disposable paper towels
alcohol swabs	biohazard disposal containers

Supplied by Coordinating Center

vial labels
specimen shippers

Supplied by Central Laboratory

7-mL royal blue top trace metal Vacutainers
15-mL red top Vacutainers
white filter 13-mm serum separators, in resealable bags
2 mL high-density polypropylene storage vials
250- μ l and 1.0-mL micropipettors and tips
Pre-weighed vials containing 3 g metaphosphoric acid

2. PARTICIPANT PREPARATION (Section 18.5)

! Fasting for at least 4 hours
! Resting in seated position for at least 5 minutes
! No smoking, exposure to cold, or exertion for at least 30 minutes
! Clothing should not restrict arm

3. BLOOD COLLECTION (Section 18.5)

Collect and completely fill, **in this order:**
1 15-mL red-top Vacutainer
1 7-mL royal blue-top Vacutainer

4. EXTRACTING SERUM (Section 18.6)

Centrifuge for 15 minutes at 1500 x G

5. SPECIMEN PROCESSING AND STORAGE (Section 18.7)

Prepare: Vial 1 - serum for cholesterol/triglycerides
Vial 2 - serum for HDL cholesterol
Vial 3 - serum for zinc/copper
Vial 4 - serum for vitamin A/E/carotenes
Vial 5 - serum-extract for vitamin C
Vial 6 - excess serum for storage

6. SHIPPING (Section 18.8)

Use specimen shippers to send to CDC via Federal Express (include Specimen Shipping Manifest in mailer)
Send copy of Manifest to CDC via Fax
By each participant's ID, neatly place a bar-coded label for ID verification upon receipt.
Retain copy of Manifest at Clinical Center

EXHIBIT 18-2. SUPPLEMENTAL SHIPPING LABEL

**Exhibit 18-3. CENTRAL LABORATORY REFERENCE RANGES FOR SUBJECTS
AGES 55 YEARS AND OLDER**

Analyte/ Subjects (n)	Mean	5th Percentile	95th Percentile	Reference
Retinol, µg/dL (5,867)	64	39	93	1
Vitamin E, µg/dL (5,867)	1,405	775	2,489	1
Vitamin C, mg/dL Men (2,775) Women (2,924)	0.75 0.95	0.07 0.13	1.49 1.79	1
Beta-carotene, µg/dL Men (2,857) Women (3,010)	22 30	4 6	56 73	1
Lutein/zeaxanthin, µg/dL (5,867)	25	9	47	1
Beta-cryptoxanthin, µg/dL (5,866)	10	2	23	1
Lycopene, µg/dL (5,867)	19	4	41	1
Alpha-carotene, µg/dL (5,867)	6	0.3	14	1
Total Cholesterol, mg/dL Men (2,873) Women (3,029)	213 233	150 166	281 307	1
HDL-cholesterol, mg/dL Men (2,857) Women (3,014)	45.4 56.0	27.6 32.2	71.4 85.8	1
Total Triglyceride, mg/dL Men (2,869) Women (3,025)	168 166	62 64	366 368	1
Serum zinc, µg/dL Men (2,070) Women (2,353)	87.0 83.8	64.6 64.6	110.6 104.0	2-3
Serum copper, µg/dL Men (2,065) Women (2,342)	119.5 135.0	88.1 100.4	157.6 182.7	2-3

(See Notes for Exhibit 18-3 on following page).

REFERENCES

- Nutritional Biochemistry Branch, Centers for Disease Control and Prevention, Atlanta, Georgia. Unpublished data from NHANES III.

2. Gunter EW, Turner WE, Neese JW, Bayse DD. Laboratory procedures used by the Clinical Chemistry Division, Centers for Disease Control, for the Second Health and Nutrition Examination Survey (HANES II), 1976-1980. Atlanta: Centers for Disease Control, 1981. (Revised edition, 1985)
3. Fulwood R, Johnson CL, Bryner JD, Gunter EW, McGrath CR. Hematological and nutritional biochemistry reference data for persons 6 months - 74 years of age: United States 1976-1980. Vital and Health Statistics, Series II, No. 232. DHHS Publication No. (PHS) 83-1682, Washington, DC: U.S. Government Printing Office, 1982.

Notes for Exhibit 18-3.

1. Nationally representative data from the Third National Health and Nutrition Examination Survey (NHANES III) are used in interpreting baseline values from subjects in the Age-Related Eye Disease Study (AREDS). The vitamin data are not to be cited or distributed without permission from the AREDS Central Laboratory, Nutritional Biochemistry Branch, Centers for Disease Control and Prevention, Atlanta, Georgia.
2. Serum zinc and copper were not measured in NHANES III; therefore, the values from NHANES II are listed. From the NHANES II data, it is evident that serum zinc concentrations vary diurnally and with food consumption.²⁻³ Criteria established for a low serum zinc are as follows: (p. 1395)³ “<70 µg/dL for persons sampled in the morning after an overnight fast; <65 µg/dL for persons sampled in the morning, but not requested to fast; and <60 µg/dL for persons sampled after noon and not requested to fast.” Applying these criteria to the NHANES II dataset, 1.4-3.0% of persons aged 45-74 had a low serum zinc value.³ In AREDS, the fasting status and time of blood collection will be important for interpretation of the zinc data.
3. There are no recognized “toxic” values for any of these analytes. For alert values, a total serum cholesterol concentration of ≥300 mg/dL or an HDL-cholesterol concentration of ≤30 mg/dL may be used. In general, a serum vitamin A concentration >150 µg/dL in combination with elevated serum retinyl esters would be consistent with vitamin A toxicity.
4. A serum triglyceride concentration of ≥300 mg/dL will be considered strongly indicative of a lipid disorder and/or noncompliance with the dietary instructions. In this situation, the corresponding lipoprotein data should be interpreted with caution.