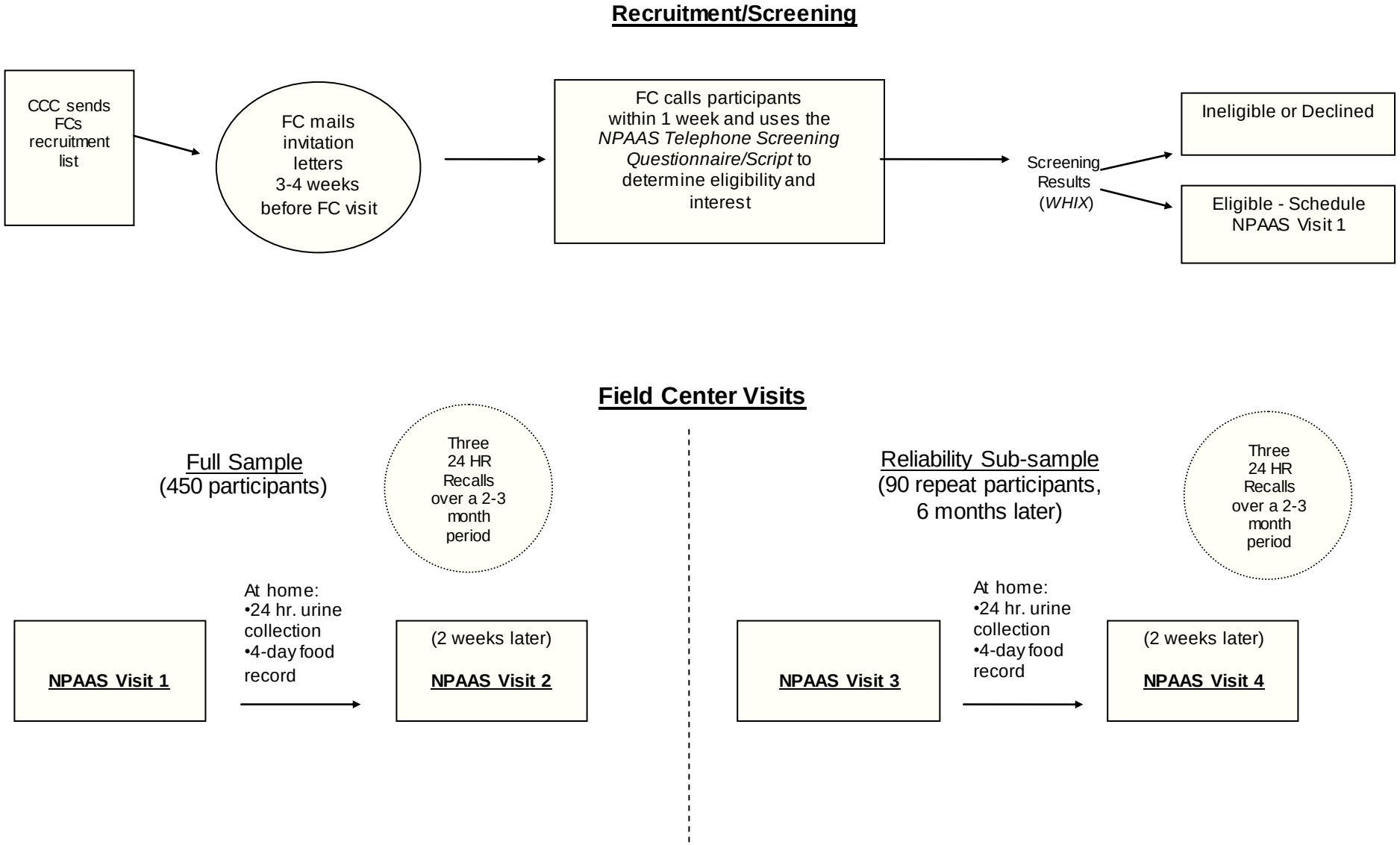
SECTION 1
WHI EXTENSION STUDY NUTRITION AND PHYSICAL ACTIVITY ASSESSMENT STUDY OVERVIEW

1.0 Overview of the WHI -ES Nutrition and Physical Activity Assessment Study (NPAAS)

- The WHI Extension Study (WHI-ES) Nutrition and Physical Activity Assessment Study (NPAAS) is a substudy of the WHI Extension Study. The objective of the NPAAS is to collect biological markers of dietary intake (energy, protein, fatty acids, and micronutrients) and physical activity for use in regression calibration models that will correct the random and systematic bias of dietary and physical activity self-report and better estimate associations of dietary intake and physical activity with disease outcomes. The calibrated nutrient intake data will be used for a broad range of nutrient-disease and phyisical activity-disease analyses from the WHI. For a copy of the NPAAS protocol, refer to *NPAAS Manual, Appendix A – NPAAS Protocol (Ver.2.1)*.

- NPAAS will be implemented in a subset of 450 women in the WHI Observational Study who have consented to participate in the WHI Extension Study. Women will be mailed an invitation letter prior to being contacted for a telephone screening. Willing participants will be consented at the first NPAAS clinic visit, which lasts about 5 hours. Following consent, they will complete an FFQ, provide information about current dietary supplements (*Form 45*) and physical activity (*Form 35 and Arizona Activity Frequency Questionnaire or Seven-Day Physical Activity Recall*), complete a psychosocial questionnaire (*Form 171- Viewpoints*), receive a single oral dose of doubly labeled water (DLW), and provide four spot urines. Women aged 60 years and older will also provide a blood specimen. Women will receive instructions and view a video about the correct way to keep a four day food record. Between clinic visits women will record all foods and beverages for four days using a standardized food record booklet, and they will collect a 24-hour urine specimen. Women will return to their Field Center for a 3-hour visit on about Day 15, at which time they will return their completed *Four-Day Food Record* and the 24-hour urine specimen (collected at home), be weighed, provide a fasting blood specimen, complete an indirect calorimetry protocol, give two spot urines, and complete either the *Arizona Activity Frequency Questionnaire* or *Seven-Day Physical Activity Recall*, whichever one they did not complete at visit 1. All women will complete three 24-hour dietary recalls after the second clinic visit. The first one will be completed about 2-3 weeks after visit 2 and then one a month until they are completed. For a subset of 90 women (the reliability subsample), the full protocol will be repeated approximately 6 months later. The Nutrition and Physical Activity Assessment Study implementation at the FCs begins January 2007 and continues through June 2008 Figure 1.1 – *WHI Extension Study (WHI-ES) Nutrition and Physical Activity Assessment Study Overview* provides an overview of NPAAS.

Figure 1.1
WHI-ES Nutrition and Physical Activity Assessment Study Overview



SECTION 2
GUIDELINES

2.0 Overview

- This section contains information to help Field Centers (FCs) manage the Nutrition and Physical Activity Assessment Study. Guidelines for staffing, training and communication, and supplies are provided. This section may be helpful for staff training and can be used as a checklist to ensure that essential elements are in place to conduct the study.

2.1 Staffing

- Each FC has the following staff positions covered in their initial NPAAS budget projections: Project Coordinator, Nutritionist, Medical Assistant, Research Assistant, and Lab Technician, or similar configurations to support implementation of the NPAAS at each FC.

2.1.1 NPAAS Lead-Ops

- Each FC identifies an NPAAS Lead-Ops person to oversee NPAAS operations and be the NPAAS point person. This person may be the FC Principal Investigator, the investigator with local oversight for the NPAAS, or a separate person. The NPAAS Lead-Ops person needs to:
  - Be in the clinic and involved in the NPAAS visits so that they can supervise what is happening and respond to questions that staff may ask.
  - Train other non-lead NPAAS staff.
  - Ensure that the NPAAS protocol is followed, supplies are available, specimens and completed forms are shipped according to the schedule provided by the CCC and any procedural problems or adverse events are reported to the CCC.
  - Oversee management of the following NPAAS operations:
    - Recruitment
    - NPAAS visits and tasks
    - FC-NPAAS budget oversight
    - FC IRB submissions and renewals
    - Other duties as required

2.2 Training

- The purpose of training within NPAAS is to teach staff the NPAAS protocol and procedures for implementation at the Field Center (FC). To facilitate the implementation of NPAAS, the CCC uses a train-the-trainer model similar to the one used in WHI. One NPAAS Lead-Ops person per FC receives training for all NPAAS-specific tasks and is then responsible for training other staff for NPAAS tasks. Staff who are responsible for administering the doubly-labeled water, indirect calorimetry, and phlebotomy tasks are strongly encouraged to attend the appropriate training components provided on-site by the CCC NPAAS Project Coordinator. The CCC provides one time FC on-site training, conference call training, and the *NPAAS Manual* (refer to *NPAAS Manual, Appendix E – NPAAS Training Materials*). The FC NPAAS Lead-Ops is responsible for training of the NPAAS to staff who are unable to attend the on-site training. Additional or follow-up training may occur through conference calls.

2.2.1 NPAAS Staff

NPAAS Lead-Ops

- The NPAAS Lead-Ops person attends the on-site FC NPAAS training conducted by the CCC NPAAS Project Coordinator at his/her FC and reads the *NPAAS Manual*.

Non-Lead NPAAS Staff

- Non-lead NPAAS staff attend pertinent sections of the FC on-site training conducted by the CCC NPAAS Project Coordinator, read relevant sections of the *NPAAS Manual* and discuss relevant training topics with the NPAAS Lead-Ops person.

- FC staff will be trained by the CCC NPAAS Project Coordinator or FC Lead-Ops on implementing the NPAAS protocol.

- For training on NPAAS forms collection and visit implementation, including blood and urine collection, the NPAAS Lead-Ops person attends the NPAAS training and reads the *NPAAS Manual*. The Lead-Ops person trains the designated FC staff. FCs must also follow the pertinent safety precautions found in the *NPAAS Manual, Section 7.1.1– Safety Procedures – Precautions for Handling Blood and Section 7.1.2– Safety Procedures – Precautions for Handling Urine*, as well as their local regulations for bio-specimen handling (blood and urine).

2.2.2 Training Resources

2.2.2.1 NPAAS Training at Field Centers

- The CCC conducts one on-site NPAAS training for each FC at the time of NPAAS start-up.

- The *NPAAS Manual, Appendix E – NPAAS Training Materials* includes the agenda and relevant materials.

2.2.2.2 NPAAS Procedure Manual (referred to as the *NPAAS Manual*)

- The *NPAAS Manual* describes the study design (as outlined in the study protocol), procedures, and data management. The primary function of the manual is to provide common training and reference materials across all participating NPAAS FCs as a way of assuring uniform application of the protocol.

2.2.2.3 NPAAS Conference calls

- Conference calls will be used to supplement or follow-up the FC on-site training.

2.3 Communication

2.3.1 Email Communication

- The following types of email communication are sent to the FC PI, all NPAAS FC investigators, and individuals identified to receive NPAAS mailings (i.e., NPAAS Lead-Ops person).
  - **NPAAS Updates:** An email newsletter sent to FC PIs and Lead-Ops persons every other week to maintain systematic communication and keep NPAAS FCs informed.
  - **NPAAS Alerts**: Priority email sent when there is immediate information that is important to quickly relay to all NPAAS FCs.

- FCs send NPAAS-related questions to the CCC NPAAS Project Coordinator Lynn Fleckenstein (lflecken@whi.org) for triaging to the appropriate CCC staff for a response.

2.4 Print Materials and Supplies

- This section provides information about print materials and supplies for the Nutrition and Physical Activity Assessment Study (NPAAS).

2.4.1 Print Materials

2.4.1.1 Materials Printed by Field Centers (FCs)

- FCs print several of the NPAAS forms and materials locally from masters provided by CCC. Electronic copies of the masters are provided to the FCs. Hard copies of the masters are also available in the *NPAAS Manual (Section 9 – NPAAS Forms and Worksheets, Appendix C – NPAAS Participant Materials* and *Appendix D – NPAAS Staff Materials)*. The NPAAS forms and materials that FCs print locally are outlined below.
  - Letter of Invitation to Participants (to be printed on local institutional stationary)
  - Telephone Screening Questionnaire/Script
  - Frequently Asked Questions (for staff)
  - Study at a Glance
  - NPAAS Consent
  - Instructions for 24-hour Urine Collection
  - Record Sheet for 24-Hour Urine Collection
  - NPAAS 24-Hour Urine Collection Worksheet for Staff
  - Between Visits At A Glance
  - Four Day Food Record Worksheet for Staff
  - NPAAS Visit Forms (Forms 171-172, 174-178)
  - NPAAS Visit 1 Eligibility Worksheet
  - NPAAS Visit 2 Participant Update Worksheet
  - NPAAS Pre-Visit 3 Eligibility Worksheet
  - NPAAS Visit 3 Eligibility Worksheet
  - NPAAS Visit 4 Participant Update Worksheet
  - Seven Day Physical Activity Recall (PAR) Script
  - Mailing Labels
  - Return Address Labels
  - Participant Member ID Labels
  - Resting Energy Expenditure Results Letter
  - 24 Hour Recall Notification Letter
  - Consent for Future Contact

2.4.1.2 Print Materials Supplied by the CCC

- The CCC provides each FC with the print materials outlined below.
  - NPAAS label set for lab specimens, activity questionnaires, and indirect calorimetry
  - Form 35 – Personal Habits Update
  - Form 45 (Back-up) – Current Supplements (Back-up)
  - Form 60 – Food Frequency Questionnaire
  - Form 61 – Food Questionnaire Instructions
  - Four-Day Food Record booklet
  - Portion size booklet for use in completing the Four-Day Food Record and 24-hour recalls
  - Arizona Activity Frequency Questionnaire

2.4.2 Supplies and Guidelines for Ordering Supplies

The list below includes the list of NPAAS supplies and guidelines for ordering them. FCs may choose to use a different vendor than the one recommended for supplies obtained locally.

Supplies	Recommended Vendor/Part Number:	Source
Doubly labeled water (DLW) protocol		
• DLW (in pre-measured bottles)	Dr. Dale Schoeller, University of Wisconsin	Schoeller lab
• Pre-weighed tissues (for use in case of DLW spillage)	Dr. Dale Schoeller, University of Wisconsin	Schoeller lab
Miscellaneous		
• Participant meal replacement beverage (8 ounce) for 1 hour after DLW dose at Visit 1 1 per NPAAS participant	Local medical supply or store Suggested brand: Boost (based on informal taste testing at the CCC)	FC
• Participant lunch at the end of Visit 1 1 per NPAAS participant	FC option	FC
• Participant snack at the end of Visit 2 1 per NPAAS participant	FC option	FC
• Straws (for drinking DLW) 2 per NPAAS participant	Local medical supply or store (a small size that can be pushed all the way inside the DLW dose bottle)	FC
• Ziplock baggies (extras for use as needed with pre-weighed tissues in case of DLW spillage) 1 box (of ~50) per FC	Local store	FC
• Office supplies, e.g., envelopes, mailing labels, member ID labels, postage	Local store	FC
• Measuring cups and spoons for completing the 4DFR 1 set per participant	CCC will order and provide	CCC
• WHI #2 Pencils – 1 per participant	CCC will provide	CCC
• WHI Folders – 1 per participant	CCC will provide	CCC
Blood and Urine Collection Supplies		
• Urine collection hats (Specipans) 3 per NPAAS participant – 1 to use at each of two clinic visits, 1 to send home with participant for 24-hour urine collection	CCC will order and provide	CCC
• 24-hour urine collection containers 2 (3 liter) per NPAAS participant	CCC will order and provide	CCC
• Plastic funnels for 24-hour urine collection and processing 1 per NPAAS participant for at-home collection Extras as needed for pouring contents of two collection bottles together for mixing; Needed for large collections (i.e. > 3L)	CCC will order and provide	CCC

• Plastic carry bag for 24-hour urine collection kit 1 per NPAAS participant	CCC will order and provide	CCC
• Plastic container (with lid) for mixing large urine collections (2.5 gallon)	CCC will order and provide	CCC
• Safety pins (large size) for participants to pin to their undergarments as a reminder for 24-hour urine collection 1 per NPAAS participant (1 box of ~100 per FC)	Local store	FC
• Gel ice packs for participants to transport their 24-hour urine collections to the Field Center 20 per FC (~6 ounce; 4" x 6")	Local medical supply or store	FC
• PABA (B-vitamin) tablets, 3 per participant	CCC will order and provide	CCC
• Boric acid powder, one 500 gram jar, for urine collection preservative	CCC will order and provide	CCC
• Stickers for 24-hour urine collection containers	CCC will order and provide	CCC
• Royal blue-stoppered hematology (serum) tubes with Hemogard closure and clot activator, **6 mL** 3 per NPAAS participant	Fisher Catalog #14-816-154 BD Vacutainer Labware No.: 368380	FC
• Lavender-stoppered plasma 10 mL tube, with **powdered Sodium (Na_2 EDTA)** 1 per NPAAS participant under the age of 60 years 2 per NPAAS participant 60 years of age and older	Fisher or Monoject 310745 Terumo Venoject T200SQ (Baxter B3042-54 or Laboratory Supply)	FC
• Needles, 21 gauge, 1 to 1½" multiple sample Vacutainer®	Local medical supply	FC
• 23-gauge butterfly with 12" tubing with multiple sample Luer adapter	Local medical supply	FC
• Syringes	Local medical supply	FC
• Monoject hypodermic needle (21-23 gauge, 1 ½")	Local medical supply	FC
• Blood draw workstation	Recommended Baxter S9267-1	FC
• Vacutainer® holder	Local medical supply	FC
• Test tube racks	Local medical supply	FC
• Alcohol swabs or cotton balls, alcohol, and alcohol dispenser or gauze swabs	Local medical supply	FC
• Bandages ("Band-Aids") or surgical tape	Local medical supply	FC
• Biohazard container for needles ("sharps" container)	Local medical supply	FC
• Biohazard container for waste	Local medical supply	FC

• Wash bottle	Local medical supply	FC
• Lab coat	Local medical supply	FC
• Disposable latex gloves (or substitute)	Local medical supply	FC
• Anti-bacterial hand soap	Local store	FC
• Aluminum foil or yellow plastic sleeves to protect the blood drawn in **all** of the fasting blood collection tubes from light (NPAAS Visit 2 & NPAAS Visit 4)	Local store	FC
Blood and Urine Processing Supplies	**Recommended Vendor/Part Number:**	
• 5 mL Corning cryovial tubes with rubber O-ring for processing spot urine samples 6 per NPAAS participant 12 additional for each participant who has two additional aliquots taken at each time point for QC	CCC will order and provide	CCC
• 10 mL tube for centrifuging 24-hour urine collection samples 2 per participant per FC plus 20 extra 4 additional for each participant in the QC sample	CCC will order and provide	CCC
• Disposable transfer pipettes (for transferring urine from DLW spot urine collection to 5 mL cryovial, and for transferring the centrifuged 24-hour urine sample into the two 2 mL and two 5 mL cryovials) 7 per participant	CCC will order and provide	CCC
• 2 mL cryovials for serum, plasma and 24-hour urine, 12 total: Visit 1 3 for Lavender tube 3-hour post-DLW blood draw (for women 60 years and older) Visit 2 4 for Royal Blue fasting blood 3 for Lavender fasting blood 2 for 24-hour urine (4 additional for each participant in the QC sample)	Local medical supply	FC
• 5 mL cryovials with screw cap for 24-hour urine 2 per participant 4 additional for each participant in the QC sample	CCC will order and provide	CCC
• Toploading Digital Scale (for measuring weight of 24-hour urine collections)	CCC will order and provide, where necessary	CCC
• Fluid-resistant lab coat	Local medical supply	FC
• Refrigerated centrifuge with swinging buckets (able to reach relative centrifugal force of 1,300 xg)	Local medical supply	FC

• Factory certified low temperature thermometer, -90°C to +20°C or thermistor		FC
• Large and small test tube racks for holding Vacutainer® tubes and cryovials	Local medical supply	FC
• Disposable latex gloves (or substitute)	Local medical supply	FC
• Chlorine bleach	Local medical supply	FC
• Goggles or glasses or mask with face shield or barrier shield behind which to process blood samples	Local medical supply	FC
• Tape	Local store	FC
• 1 mL adjustable automatic pipettor	Local medical supply	FC
• Disposable pipette tips (for blood processing)	Local medical supply	FC

Blood and Urine Storage and Shipment

• Freezer boxes for 5 mL Corning cryovial tubes	CCC will order and provide	CCC
• Freezer at -70°C or colder, with CO_2 back-up system and temperature recorder		FC
• Factory certified low temperature thermometer, -90°C to +20°C or thermistor		FC
• Suggested shipping supplies: (Please refer to your local institution's shipping guidelines)		FC
o Insulated Shippers	FFisher Catalog # 03-530-17 (SCA THERMOSAFE No.:355) **or**Fisher Catalog #03-530-32 (SCA THERMOSAFE No.:398)	FC
o Freezer alarm for each -70°C freezer		FC
o Waterproof packing tape (strapping tape)		FC
o Dry ice nuggets		FC
o Indelible ink pen		FC
o Shipping tape		FC
o Scale for weighing shipment		FC
o Bubble wrap (to cushion cryovials inside freezer box)		FC
o Paper towels (for freezer box exterior; to absorb condensation)		FC
o Leak-proof plastic bag		FC
o Maps for freezer boxes		FC
o Notification of NPAAS Shipment Form		FC
o Frozen Specimen Shipping Labels		FC
• Other supplies as needed for safe handling of biospecimens		FC

SECTION 3
RECRUITMENT

3.0 Overview

- This section contains information to help FCs manage NPAAS recruitment. The materials describe the NPAAS study sample, recruitment goals, recruitment plan, recruitment list, invitation letter, and resources for recruitment.

3.1 NPAAS Study Sample

- NPAAS will be implemented in a subset of 450 WHI Observational Study (OS) women who have consented to participate in the WHI Extension Study.

3.2 Recruitment Goals

- The recruitment goals for each FC are as follows:
  Chapel Hill, Madison, Memphis, New York, Tucson, and Worcester – 40 participants each
  Chicago, Oakland, and Seattle – 70 participants each

- The three minority FC sites are Memphis, New York, and Tucson, which are expected to recruit only minority participants - a total of 120 women from racial/ethnic minorities.

3.3 Recruitment Plan

- Each FC receives an NPAAS recruitment plan from the CCC based on weighted sampling for age, body mass index and minority status, in addition to recruitment criteria specified in Section 3.4. *Recruitment Lists* of the NPAAS Procedure Manual.

- FCs will explicitly follow the NPAAS recruitment plan. The CCC will monitor recruitment rates.

3.4 Recruitment List

- The CCC provides each FC with a list of eligible participants by way of the *Ancillary Study Recruitment Tracking* report (ANC002). The report includes OS participants who have consented to participate in and are active in the WHI Extension Study and who meet the eligibility criteria outlined in the NPAAS protocol. The list includes women who meet the following criteria:
  - Have an FFQ at baseline.
  - Participate in the OS at the full follow-up level.
  - Do <u>not</u> take insulin or oral hypoglycemic agents to manage diabetes.*
  - Do <u>not</u> require supplemental oxygen.*
  - Have <u>not</u> had blood transfusions, administration of blood products or administration of intravenous fluids in excess of 500mL in the week prior to the first clinic visit for this study or an expectation of the same during the period between visits 1 and 2 (including IV fluids administered as part of any administered anesthesia such as during a screening colonoscopy).*
  - Will remain within 200 miles of their home during the week prior to the study and throughout the two week study period. *

- Have not lost or gained more than 10% of their body weight in the previous month. *
- Do not have bladder control problems that would make collection of a 24-hour urine specimen difficult.

- Do not have self-reported claustrophobia, which could cause the participant to become anxious when the plastic hood is placed over their head for the indirect calorimetry procedures. These procedures require that the participant lie quietly for at least 30 minutes, as anxiety caused by claustrophobia would produce inaccurate test results.

* These factors interfere with the doubly labeled water measurements of energy expenditure, indirect calorimetry measures, or other study procedures.

- FCs use the *NPAAS Ancillary Study Recruitment Tracking* report (ANC002) to invite and schedule women for NPAAS. The report indicates whether women are eligible for the study at the time the report is run based their current WHIX status (e.g. deceased, absolutely no follow-up, proxy). For further details about the recruitment list and the report, refer to *NPAAS Manual, Section 3.6 - Resources for NPAAS Recruitment Tracking*.

3.5 Invitation Letter

- After receiving the participant recruitment list from the CCC, FCs begin to mail the NPAAS invitation letter to participants in weekly batches of 25. A copy of the *NPAAS Letter of Invitation to Participants* is available the *NPAAS Manual, Appendix C – NPAAS Participant Materials*.

- It is important to mail the NPAAS invitation letter separate from other WHI Extension mailings. This provides an opportunity to:
  - Ensure that the invitation letter is not lost in the WHI Extension materials.
  - Send the invitation letter in a timely manner.

- It is important to mail the invitation letter in small batches (i.e., do not to mail to the entire NPAAS recruitment list all at once). The CCC recommends batches of 25 in the early stages of recruitment. If FCs mail too far in advance, the participant's strata status may change before a FC can contact the participant.

- To avoid any potential of selection bias (subjectively selecting participants), FCs should mail an invitation letter to only the participants appearing on their *Ancillary Study Recruitment Tracking* report (i.e., don't add names) and continue to invite participants sequentially as appearing on the report unless:
  - The stratum closes before the FC approaches them.
  - The participant is known *a priori* to be ineligible (e.g., lives greater than 200 miles from the FC). Refer to *NPAAS Manual, Section 4.1.4 – Complete and Data Enter Form 174 – NPAAS Status Update*.

- To track the NPAAS mailings and screening calls, each FC sets up a system to track the following pieces of recruitment information:
  - Who has been sent the NPAAS invitation letter.
  - Date letter sent.
  - Date to call participant (one week after invitation letter sent).
  - Number of phone attempts (refer to *NPAAS Manual, Section 4.1.1 - Telephone Contact*).

3.6 Resources for NPAAS Recruitment Tracking

- This section describes the resources for NPAAS recruitment tracking. This includes the *Ancillary Study Recruitment Tracking* report and the Ancillary Study Recruitment Data Source in the WHIX Custom Data Extract (CDE) system.

3.6.1 Ancillary Study Recruitment Tracking Report

- The *Ancillary Study Recruitment Tracking* report provides the participant details necessary for tracking NPAAS recruitment.

- The *Ancillary Study Recruitment Tracking report (ANC002)* is found in the *Member/ Tracking Reports* menu in WHIX (see *Figure 3.1 – Accessing the Ancillary Study Recruitment Tracking Report in WHIX*). The report is used to show participants who are eligible for a specific ancillary study. ***This report is dependent upon ancillary study-specific recruitment data downloaded from the CCC to individual FCs.*** If no recruitment data have been downloaded to an FC's server by the CCC, the report will display no data.

- This report can be run and data displayed in a variety of ways depending upon values entered in the parameter screen. The 11 parameters for this report are described below. To run the report after entering the parameters, click the **Run Report** button.

Figure 3.1. Accessing the Ancillary Study Recruitment Tracking Report in WHIX

3.6.1.1 Parameter Definitions (refer to *Figure 3.2 – Report Parameters Screen*)

Report Format

- *List format* is set as the current default value. *List format* prints a list of approximately 8 participants per page with AS enrollment status, ID number, name, address, phone, contact notes WHI study, ethnicity, language, current age/DOB, follow-up status and AS status (based on enrollment code entered)
- *Call log* format prints one participant per page with all of the information shown for list format, and provides a space to keep track of telephone contact attempts.

Ancillary Study: This parameter is a drop down list showing ancillary studies for which recruitment data are available. Currently, only NPAAS is listed.

Participant ID: To run the report for an individual participant, enter the participant member ID number (without check digit) in this field.

Common ID: To run the report for an individual participant based on the participant's common ID number, enter a common ID number in this field.

Recruitment order: NPAAS has a pre-determined recruitment order. You can limit the participants for whom the report is run, based on recruitment order, by entering a recruitment order range in these fields. By default, the report runs for participants with recruitment order 1 through 100. **However, the CCC recommends that FCs begin with recruitment order 1 through 25.**

WHI Study: For NPAAS, FCs should use the default parameter, *All Studies*. The complete list of values appearing in the drop-down list are as follows:

- All Studies – to view eligible participants who are in all WHI study components (DM, HRT, CaD and OS) select this option.
- OS - to limit the list to only those eligible participants who are in OS, select this option.
- DM - to limit the list to only those eligible participants who are in DM, select this option (note that these participants may also be in HRT and/or CaD).
- HRT - to limit the list to only those eligible participants who are in HRT, select this option (note that these participants may also be in DM and/or CaD).
- CaD - to limit the list to only those eligible participants who are in CaD, select this option (note that these participants will also be in HRT or DM or both.)

Race/Ethnicity: This parameter is a drop down-list from which you must select one of the following values:

- All races/ethnicities – to view eligible participants of all races/ethnicities listed on *Form 2 – Eligibility Determination,* select this option. Participants with "unknown" race/ethnicity will be included. 'All races/ethnicities' is the current default value.
- All minorities – to limit the list to those eligible participants who are entered in WHIX with race/ethnicities other than *White,* select this option.
- American Indian or Alaskan native – to limit the list to those eligible participants who are entered in WHIX with the race *American Indian or Alaskan native,* select this option
- Asian or Pacific Islander - to limit the list to those eligible participants who are entered in WHIX with the race *Asian or Pacific Islander*, select this option
- Black or African American - to limit the list to those eligible participants who are entered in WHIX with the race *Black or African-American*, select this option
- Hispanic - to limit the list to those eligible participants who are entered in WHIX with the ethnicity *Hispanic*, select this option

- White - to limit the list to those eligible participants who are entered in WHIX with the race *White*, select this option
- Other - to limit the list to those eligible participants who are entered in WHIX with the race/ethnicity *Other*, select this option
- Unknown - to limit the list to those eligible participants who are entered in WHIX with no race or ethnicity, select this option

Current Age between ___ and ___: This parameter allows you to specify a range of eligible participants to appear on the list based on current age. Enter the lower age in the range in the first field and the upper age in the second field. Leaving these fields blank will yield participants of all ages.

Ancillary study enrollment status: This parameter is a drop down list that allows you to run the report to show participants who are enrolled in an ancillary study or not enrolled or both.

- All enrollment statuses – choose this option to see both those participants who are enrolled in the ancillary study and those who are not enrolled. This is currently the default parameter.
- Enrolled – choose this option to see eligible participants who have an ancillary study consent task entered in WHIX with a status indicating the participant is enrolled in the study. Note that more than one status may be associated with a participant being enrolled in the study.
- Not Enrolled – choose this option to see eligible participants who either do not have an ancillary study consent task entered in for the ancillary study, or have an ancillary consent task entered with a status indicating the participant is not enrolled in the study. Note that more than one status may be associated with a participant not being enrolled in the study.

Ancillary study participation status:

- The ancillary study participation status is the status entered for a participant's ancillary study consent task. These statuses are ancillary study specific.
- The following two parameter values will show up in the parameter drop down-list for all ancillary studies.
  - All ancillary study participation statuses. This is the current default parameter.
  - No ancillary study participation status yet
- Any other values that show in the drop down-list are ancillary study specific.

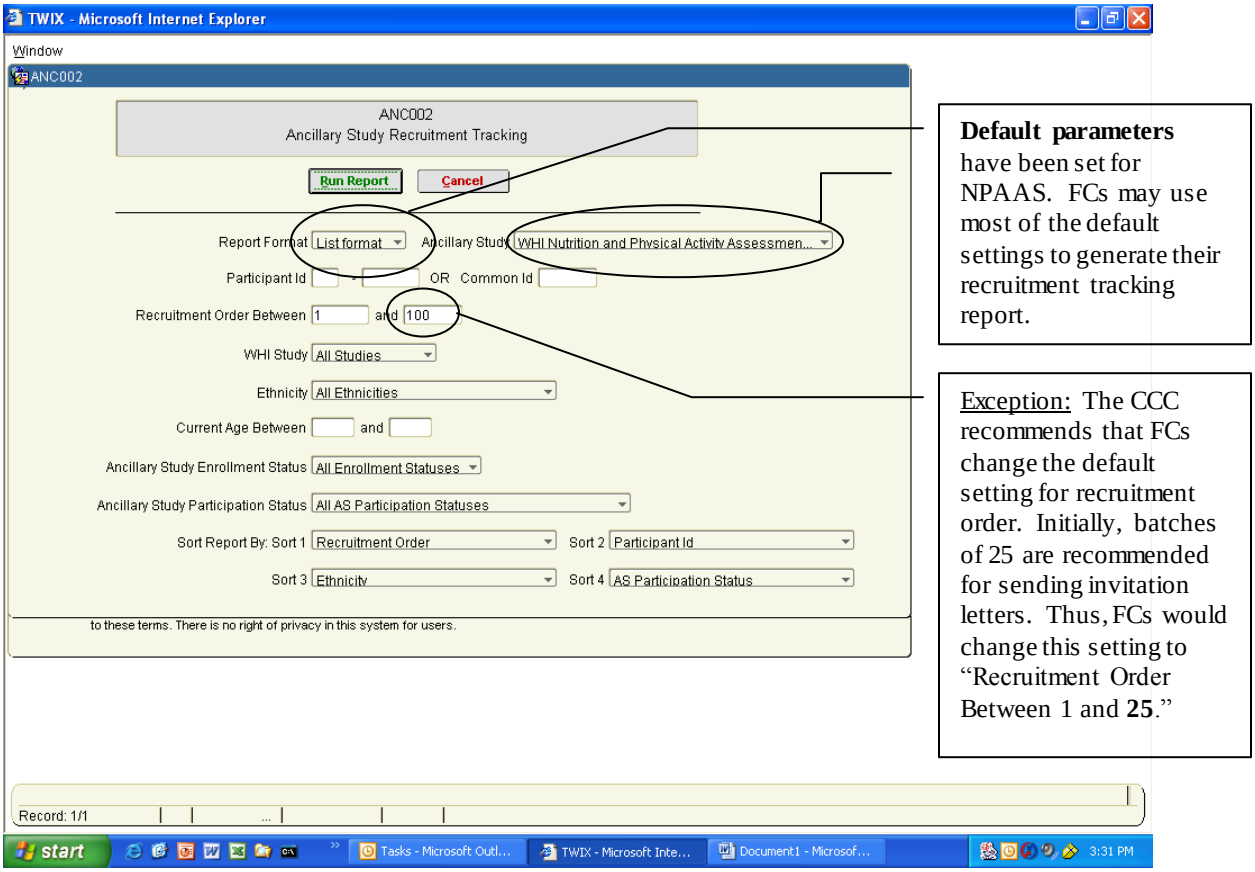
Sort by:
The report output can be sorted on 4 levels, using 7 sort variables:

- Ancillary study participation status
- Common ID
- Race/ethnicity
- Language [preferred language English or Spanish]
- Participant ID
- Participant [last] Name
- Recruitment order

There are 4 fields for entering sort variables in the parameter screen (sort 1, sort 2, sort 3, sort 4). By default, the *Sort 1* parameter has the value Recruitment Order entered and sorts the report by recruitment order. You may change the values in any of the 4 sort fields. For example, to sort the report first by preferred language, then by ethnicity, then by last name and finally by participation status you would enter the following values:

Sort 1 – Language
Sort 2 – Race/ethnicity
Sort 3 – last name
Sort 4 – AS participation status

Figure 3.2: Report Parameters Screen



Default parameters have been set for NPAAS. FCs may use most of the default settings to generate their recruitment tracking report.

Exception: The CCC recommends that FCs change the default setting for recruitment order. Initially, batches of 25 are recommended for sending invitation letters. Thus, FCs would change this setting to "Recruitment Order Between 1 and **25**."

3.6.2 Ancillary Study Recruitment Data Source in the WHIX Custom Data Extract (CDE) system

- The Custom Data Extract (CDE) system in WHIX contains a data source with the same columns as the Ancillary Study Recruitment Tracking report, plus some additional participant information from the WHIX database.

- FCs can use the CDE to generate NPAAS mailing labels and participant member ID labels for the front page of NPAAS forms. FCs familiar with the WHIX CDE system may also use it for tracking NPAAS recruitment and NPAAS visit status, if they choose.

SECTION 4
SCREENING

4.0 Overview of Screening Process

- The NPAAS screening process determines the eligibility and interest of potential WHI-OS Extension Study participants. Below is a summary overview of the screening process:
  - Contact each participant by phone within one week of mailing the invitation letter.
  - Use the *Telephone Screening Questionnaire/Script* to determine the participant's eligibility and interest.
  - Schedule NPAAS Visit 1 and Visit 2 for eligible and interested participants.

4.1 Screening Activities

4.1.1 Telephone Contact

- FCs telephone all participants who are mailed an invitation letter within one week of the NPAAS mailing. The NPAAS interviewer uses the Call Attempts Log on the *Ancillary Study Recruitment Tracking* report or a similar FC-designed Call Attempts Log to record the dates when a participant is contacted by phone.
  - The CCC strongly recommends that a FC-designed Call Attempts Log contain the following information: date, time of day, day of the week, and a place for interviewer's comments.

- If the NPAAS interviewer is unable to reach the participant by telephone, after at least five attempts at different times of the day and days of the week the participant is marked '4 – Unable to contact' on Form 174 – NPAAS Status Update (refer to *NPAAS Manual, Section 4.1.4 – Complete and Data Enter Form 174 - NPAAS Status Update*.

4.1.2 Determine Eligibility and Interest

- During the screening phone call, the NPAAS interviewer uses the *Telephone Screening Questionnaire/Script* to explain the NPAAS study and determine the participant's eligibility and interest. If needed, the interviewer may refer to *Frequently Asked Questions* document when answering participants' questions about the doubly labeled water (DLW) protocol. Copies of the *Telephone Screening Questionnaire/Script* and the *Frequently Asked Questions* are located in the *NPAAS Manual, Appendix D – NPAAS Staff Materials*.

- The NPAAS interviewer completes *Form 174 – Participant Status Update* (refer to *NPAAS Manual, Section 4.1.4 – Complete and Data Enter Form 174 - Participant Status Update)*.

4.1.3 Schedule NPAAS Visit 1 and Visit 2

- If a participant is eligible and interested, the NPAAS interviewer schedules the NPAAS Visit 1 and Visit 2. Visit 2 should be scheduled to occur fifteen days (two weeks) after Visit 1.

4.1.4 Complete and Data Enter Form 174 - NPAAS Status Update

- The NPAAS interviewer completes *Form 174 - NPAAS Status Update* for all participants mailed the NPAAS invitation letter. The form is entered into the WHIX systemaccording to the instructions on the forms instructions. The "Status Result" on Form 174 represents the participant's status at the end of the recruitment/screening phone call and uses the following codes :

Code	Status Result	Interpretation
1	Scheduled for Visit 1	Enrolled
2	Pending Scheduling	Enrolled
3	Ineligible	Not Enrolled
4	Declined	Not Enrolled
5	Unable to contact	Not Enrolled
6	Visit 1 complete	Enrolled **(Activates Scheduling of 24-Hour Recalls)**
7	Visit 2 complete	Enrolled
8	Visit 3 complete	Enrolled **(Activates Scheduling of 24-Hour Recalls)**
9	Visit 4 complete	Enrolled
10	Withdrawn	Not Enrolled

- There are a <u>few</u> circumstances where the FC has the option to *complete Form 174 – NPAAS Status Update* for a participant without mailing the NPAAS invitation letter and without conducting the *NPAAS Telephone Screening Questionnaire/Script*.

  - <u>Circumstance #1:</u>

    The FC knows <u>with certainty</u> that the participant will be ineligible per the eligibility questions on the *NPAAS Telephone Screening Questionnaire/Script* (e.g., the participant has moved away from the FC and would need to travel more than 200 miles for the NPAAS visit). In this case, the FC has the option to complete Form 174 with Status Result = 3 (Ineligible) without mailing the invitation letter or conducting the *NPAAS Telephone Screening Questionnaire/Script*.

  - <u>Circumstance #2:</u>

    For participants who have significant cognitive challenges (e.g., a diagnosis of dementia) or whose WHI- Extension participation may be jeopardized by receiving an NPAAS invitation letter, FCs have the option to complete complete Form 174 with Status Result = 3 (Ineligible) without mailing the invitation letter or conducting the *NPAAS Telephone Screening Questionnaire/Script*.

    Caution: please exercise this discretionary removal of potentially eligible participants only when absolutely necessary. It is important that we avoid potential bias in our sample by not 'cherry picking' and by giving each participant the benefit of the doubt and the opportunity to self-select 'declined' participation.

  - <u>In either Circumstance #1 or Circumstance #2,</u> remove participants as their names come up in recruitment batches, not all at once.

SECTION 5
NPAAS VISITS

5.0 Overview of NPAAS Visits

- Phase I (Primary Sample) includes two NPAAS visits (NPAAS Visit 1 and NPAAS Visit 2). *Figures 5.1* – Overview of NPAAS Visit 1 and *5.3* – Overview of NPAAS Visit 2 hightlight the specific tasks for NPAAS Visit 1 and NPAAS Visit 2. *Sections 5.1 - NPAAS Visit 1 (Day 1)* and *5.2 – NPAAS Visit 2 (Day 15)* describe the activities of the two primary visits in detail and follow the flow depicted in *Figure 5.1* and *Figure 5.3*.

- Phase II (Reliability Subsample) will include two additional visits (NPAAS Visit 3 and NPAAS Visit 4) for a subset of participants. Procedures for NPAAS Visit 3 and NPAAS Visit 4 will be similar to Visits 1 and 2, respectively. The purpose of the additional visits among the subset of women is to obtain measures of reliability. Refer to *NPAAS Manual, Section 6 for specific information about the reliability subsample.*

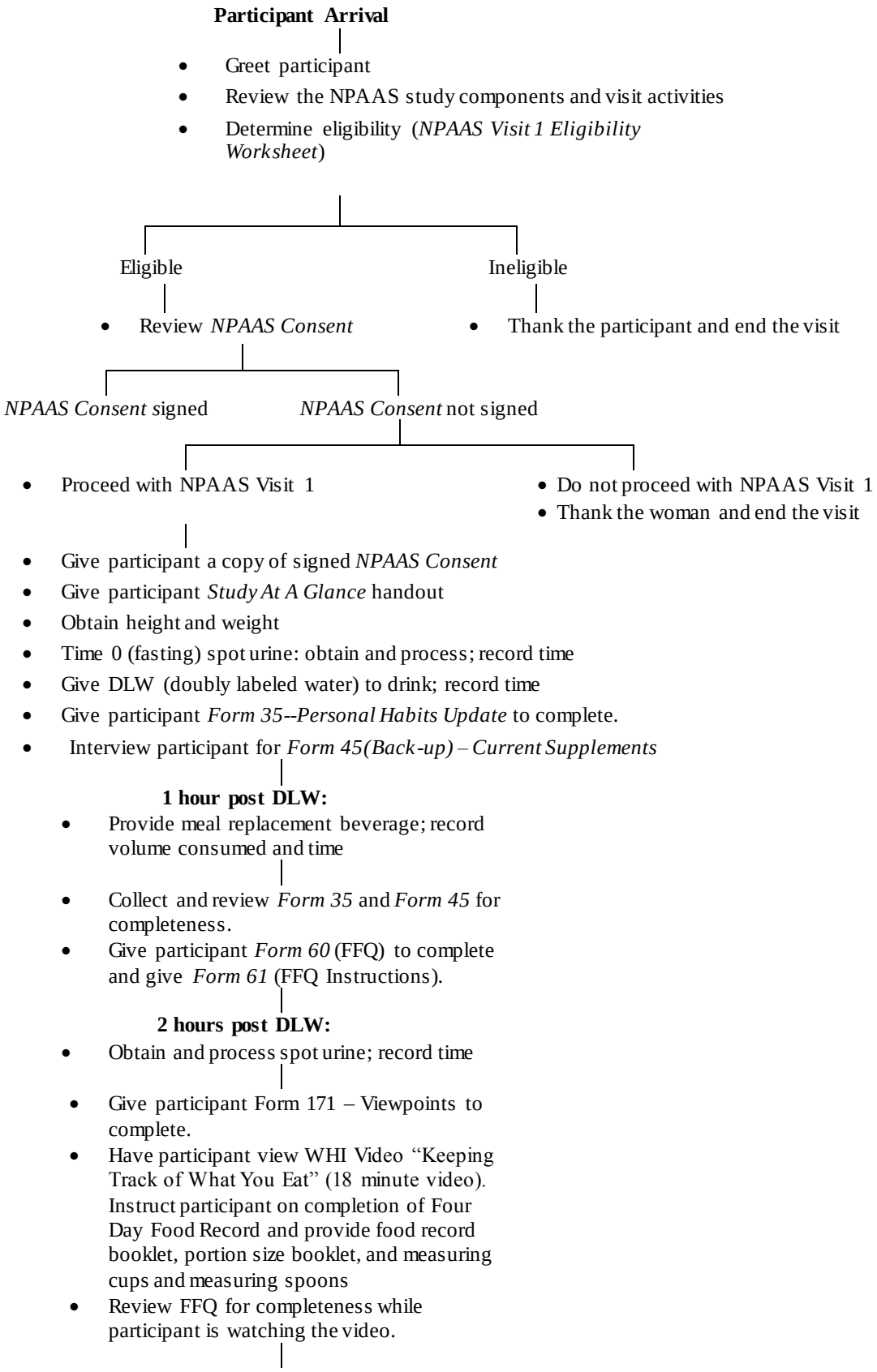
- In general, all visits contain similar activities in terms of preparation for the visit, greeting the participant, performing various visit activities, reviewing forms, providing the participant with appropriate materials before she leaves, scheduling or confirming the next visit, and shipment of forms to the CCC and specimens to Fisher BioServices. Because the visits contain many different activities and the participant will most likely be seen by several different FC staff and/or GCRC staff, it is critical to designate an NPAAS staff person (NPAAS Lead-Ops) to oversee the NPAAS visits (including pre- and post-visit activities).

5.1 NPAAS Visit 1 (Day 1)

5.1.1 Overview of Activities

- *Figure 5.1 – Overview of NPAAS Visit 1 (Day 1)* provides an overview of NPAAS Visit 1.

Figure 5.1
Overview of NPAAS Visit 1 (Day 1)



3 hours post DLW:

- Obtain and process spot urine; record time
- Obtain and process blood for women ≥ 60 years of age; record time

|

- Give participant AAFQ to complete or interview participant for *Form 172 (PAR)* (based on random assignment).
- Collect, review and review all questionnaires **before participant leaves the clinic**

|

4 hours post DLW:

- Obtain and process spot urine; record time
- Provide participant with meal/snack

|

- Instruct participant on 24-hour urine collection and provide urine collection kit
- Review 4DFR instructions with the participant
- Give participant the *Between Visits at a Glance* Sheet
- Answer any questions about between visit tasks or indirect calorimetry to be completed at Visit 2.
- Schedule or confirm participant for NPAAS Visit 2 (15 days later)
- Thank participant and escort her to the exit.

|

- Complete blood and urine processing and storage; ensure correct labels are on each specimen
- Collate all NPAAS forms into participant's NPAAS folder for use at NPAAS Visit 2.
- Complete NPAAS Status Update (Task 174) in WHIX to indicate completion of Visit 1.

5.1.2 Timing and Sequence of NPAAS Visit 1 Tasks

- It is important to maintain the sequence of NPAAS activities throughout the visit. This is important because:
  - The length of time a participant is in the clinic depends on when the NPAAS activities begin.
  - The sooner the NPAAS activities begin, the sooner the participant completes her clinic visit.
  - The sequence of the NPAAS activities related to the doubly labeled water dosing and urine/blood collections is critical to the protocol and should not be changed.

- The NPAAS protocol will take approximately 5 hours for participants to complete at Visit 1. Participants will be busy completing forms and watching the food record instruction video, but they may have some free time between tasks. Therefore, when scheduling participants, encourage them to bring something to keep busy (e.g., crossword puzzles, a book, knitting etc.)

- The sequence of the NPAAS activities related to the doubly labeled water and specimen collection may not be changed. It is critical to keep track of the time for NPAAS tasks. Consider using a stop watch or other reminder to document the first fasting urine sample, doubly labeled water dosing, and tasks scheduled at specific intervals thereafter (i.e., meal replacement beverage, spot urine collections, and blood draw for women $\geq$ 60 years of age).

- It is also important to have a designated NPAAS staff person oversee the pre and post NPAAS visit activities. This includes ensuring that all forms required by the NPAAS protocol are collected and reviewed, before the participant leaves the clinic.

Figure 5.2 – NPAAS Visit 1 Sequence of Tasks

* The sequence of the tasks in the starred columns may be modified to best meet your clinic's needs.

	Estimated Times	NPAAS Visit Tasks
45 min	5 min	Reception
	5 min	Complete *NPAAS Visit 1 Eligibility Worksheet*
	20 min	Complete *NPAAS Consent*
	5 min	Obtain- height and weight
	10 min	Time 0 - Fasting spot urine collection and processing
~ 45 min	5 min	Time 0 - Doubly Labeled Water Dosing –participant sits still for 15 minutes - completes forms
	15-20 min	*Personal Habits Update* (*Form 35*)
	10-20 min	*Current Supplements* *(Form 45)*
	5 min	Time 1 - Meal replacement beverage consumed: 1 hour after DLW
~ 60 min	30-45 min	*FFQ (Form 60)* – participant completes
	10 min	Time 2 – Spot urine collection and processing: 2 hours after DLW
~ 60 min	15min	*Viewpoints* (*Form 171)*
	20 min	WHI Video: "Keeping Track of What You Eat"
	10 min	Provide food record booklet and portion size booklet, and measuring cups and spoons
	10 min	Time 3 – Spot urine collection and processing: 3 hours after DLW
~ 60 min	5 min	Time 3 - Blood collection sample (non-fasting) - age 60 or older
	30-45 min	Participant completes AAFQ or Interviewer administers PAR (*Form 172*)
	10 min	Time 4 – Spot urine collection and processing - 4 hours after DLW
~ 30 min	10 min	Collect and review all self-administered questionnaires. Clarify missing information from forms with participant.
	10 min	Provide instructions and kit for 24-hour urine collection. Give participant *Between Visits At A Glance* Sheet.
	5 min	Review 4DFR instructions with participant and provide measuring cups, spoons, 4DFR, and Portion Size Booklet.
	5 min	Confirm appt. for next visit. Provide complementary meal (e.g., box lunch).

5.1.3 Pre-NPAAS Visit 1 Activities

5.1.3.1 Supplies and Forms

- NPAAS staff confirm that all supplies and forms needed for NPAAS Visit 1 are available for the participant's visit.

Supplies:

- WHI Folder (for participant handouts)
- WHI #2 Pencil (1)
- NPAAS label set (for forms and specimens)
- WHI Video "Keeping Track of What You Eat"
- Urine collection hats (2)
- Bounty® paper towels
- Doubly labeled water (DLW)
- *FAQ & Answers* sheet
- Syringes, needles, & gloves
- Lavender (**dry EDTA**) blood collection tubes (for participants ≥ 60 years of age) (2)
- Corning cryovials with rubber O-ring, 5 mL (for spot urine collections) (4)
- 2.0 mL cryovials (for blood collections) (3)
- Disposable transfer pipettes
- Ziplock bag with pre-weighed tissue
- Meal replacement beverage (e.g, Boost, Ensure Sustacal, 8 oz).
- One set of measuring cups and spoons for participant for the 4DFR activity
- 24-hour urine collection kit (*Instructions for 24-hour Urine Collection*, *Record Sheet for 24-hour Urine Collection*, urine collection hat, 2 [3-liter] containers, plastic funnel, large safety pin, and 2 gel ice packs in a plastic carrying bag)
  - The urine collection kit contains:
    - A highlighted copy of the *Instructions for 24-hour Urine Collection* where there is a reminder to "take one PABA (B-vitamin) tablet with each meal" on the day of the urine collection (use a Hi-Liter® marker to call attention to this text). **NOTE**: Participants who indicate an allergy or sensitivity to PABA will **not** receive PABA in their urine collection kits.
    - 3 sealed PABA (B-vitamin) tablets
    - 2 [3-liter] urine collection bottles in each of which you place 2.0 gram (**0.5 level measuring teaspoon**) boric acid * and on each of which you place two stickers, one that reads "Leave powder preservative inside bottle" and one that reads "Remember…take the PABA (B-vitamin) tablets". Each collection bottle should also be numbered and labeled with the participant's name. See *section 7.2.2.2.8. Instructions for the 24-hour Urine Collection*.

      Be sure to have recorded the weight of each urine collection bottle per instructions on the *NPAAS 24-Hour Urine Collection Worksheet for Staff* before giving the collection bottles to the participant.

      * The boric acid may be weighed or measured using household measuring spoons labeled, "For boric acid use only." When handling the boric acid, wear disposable gloves and preferably a disposable mask. Even though boric acid is a safe preservative, take precautions to avoid skin contact or inhalation.
- Complementary meal

Forms & Print Materials:

- *Study-at-a-Glance* Handout

- *NPAAS Visit 1 Eligibility Worksheet*
- *NPAAS Informed Consent Document*
- *Form 174 – NPAAS Status Update*
- *Form 175 - NPAAS Visit 1*
- *Form 60 – Food Frequency Questionnaire*
- *Form 61 – How to Fill Out the Food Questionnaire*
- *Form 35 – Personal Habits Update*
- *Form 45 – Current Supplements (Backup)* - (use hard copy; data entry will occur at CCC)
- *Arizona Activity Frequency Questionnaire* (AAFQ)
- *Form 171 - Viewpoints*
- *Form 172 (Seven-day Physical Activity Recall) and Script for PAR*
- *Four Day Food Record (Multiple Day Food Record)* & Portion Size Booklet (Serving Size Booklet)
- *Between Visits At A Glance* Sheet
- *NPAAS 24-Hour Urine Collection Worksheet for Staff*
- *Consent for Future Contact*

5.1.3.2 Pre-Visit 1 Reminder Call

- An NPAAS staff person calls each participant one day prior to her NPAAS Visit 1. If a participant is scheduled for Monday, the call may be made on the preceding Friday. During the call, complete the following:
    - Confirm the participant's NPAAS Visit 1 appointment.
    - Remind the participant to refrain from eating any food or drinking any calorie-containing beverages for **4 hours** prior to her clinic appointment time.
    - Let participant know that during the 4-hour fast she should take all her regular medications (including diuretics) with water and she may drink decaffeinated black coffee or herbal tea (without milk, cream or sugar). Regular (caffeinated) coffee or tea should be avoided. Encourage the participant to drink water liberally during the fast; otherwise, she may become dehydrated which can be uncomfortable for the participant and result in difficulties collecting urine and blood samples.
    - Ask the participant to bring all her dietary supplement bottles to the visit (they will be recorded on a hard copy of Form *45 – Current Supplements*).
    - Ask the participant to wear clothing which allows the sleeve to be easily raised above the elbow without constricting the blood flow to the forearm and hands, and allows for ease of multiple urine collections.
    - Optional: FCs may choose to use the Visit 1 Eligibility Worksheet during the pre-Visit reminder call, **in addition to** its administration during Visit 1. This may help FCs to avoid having a participant travel unecessarily to the visit if he/she is ineligible. Please note, however, that completion of the Visit 1 Eligibility Worksheet during the pre-Visit 1 reminder call does not eliminate the need to complete this task in-person at Visit 1.

5.1.4 NPAAS Visit 1 Activities

5.1.4.1 Reception

- When a participant first arrives at the FC for her NPAAS Visit 1, have her check-in at the reception desk. The Receptionist should:
  - Locate the participant's file.
  - Indicate a comfortable place where the participant may wait until she can be seen.
  - Immediately notify the NPAAS Lead-Ops (or designee) that the participant is waiting.

5.1.4.2 Eligibility

- Some of the NPAAS eligibility questions asked during the telephone screening call are repeated at NPAAS Visit 1 to ensure that the participant remains eligible. Begin the participant's visit by using the *NPAAS Visit 1 Eligibility Worksheet* to update a participant's continuing eligibility.
  - If a participant reports any change in the information she previously provided on the *NPAAS Telephone Screening Questionnaire/Script* that now make her ineligible (e.g., began taking insulin or oral hypoglycemics), mark her "Ineligible" on *Form 175 – NPAAS Visit 1*. Thank the participant and inform her that she is not eligible to participate. Escort participant to the facility exit. File the *NPAAS Visit 1 Eligibility Worksheet* in the participant's chart and change her status in WHIX task 174.
  - If a participant reports a change that would make her 'temporarily ineligible' (e.g., traveled more than 200 miles in past 2 weeks) for NPAAS Visit 1 and she is not willing to reschedule her NPAAS Visit 1, mark her "Ineligible" on *Form 175 – NPAAS Visit 1*. Thank the participant and file the *NPAAS Visit 1 Eligibility Worksheet* in the participant's chart. Change her status in WHIX task 174.
  - If a participant reports a change that would make her 'temporarily ineligible' (e.g., traveled more than 200 miles in past 2 weeks) for NPAAS Visit 1, but she is willing to reschedule her NPAAS Visit 1, thank the participant, reschedule her NPAAS Visit 1 for another day, and file the *NPAAS Visit 1 Eligibility Worksheet* in the participant's chart.
- If a participant remains eligible, mark "Eligible" on *Form 175 – NPAAS Visit 1* and begin the *NPAAS Consent* procedures.

5.1.4.3 NPAAS Consent

- Mailing the *NPAAS Consent* Before NPAAS Visit 1. The CCC recommends that the *NPAAS Consent* not be sent with the NPAAS invitation letter. It could be overwhelming to the participant to read the *NPAAS Consent* without having had the opportunity to discuss the NPAAS protocol with staff. If a FC wants to send the *NPAAS Consent* to interested participants, the FC could mail the consent to the participant after she has completed the NPAAS phone screening contact and is scheduled for NPAAS Visit 1.

- Reviewing the *NPAAS Consent*. Provide the participant with a copy of the *NPAAS Consent*. Review the consent with the participant after she has had ample time to read it. Ask her if she has any questions and answer her questions thoroughly. The following key points must be covered with the participant:
  - Her participation in the Nutrition and Physical Activity Assessment Study (NPAAS) is voluntary and does not affect her participation in the WHI Extension Study. She may withdraw from NPAAS at any time.
  - Any information she gives will be kept completely confidential and will be released to no one except WHI personnel and, if appropriate, authorized NHLBI or NCI staff.
  - Her responses will be added to those of other participants and only grouped information will be released. Neither her name nor any other identifying information will be released in WHI reports or publications.
  - A description of the NPAAS procedures during Visit 1 and Visit 2 (e.g., height, weight, administration of DLW, spot urines, blood draws, and indirect calorimetry).
  - A description of each procedure and any risks associated.
    - The loading dose of doubly labeled water may cause temporary vertigo. This is rare at the small tracer dose used for this study. As a precaution, however, participants should sit down when drinking the loading dose and should remain seated for 15 minutes after drinking the loading dose.
    - The blood draw may cause discomfort and a bruise at the site of the needle puncture. All efforts will be made to minimize this risk.
    - People with known allergies to PABA may get a slight rash if they take the PABA vitamin. Women who know they are allergic to PABA will not be asked to take it.
    - The participant may find it inconvenient to collect urine for a 24-hour period.
  - The participant will receive $100 after the second visit for time and travel expenses. Participants who do not return for the second visit procedures will not receive the $100.00.
  - All participants will also be asked to complete three 20-minute dietary phone interviews (24-hour recalls). These will begin after visit 2.
  - The potential of repeating the entire study about six months later to help us learn about the reliability of the measurements.

- Each FC must also follow the requirements imposed by their own Institutional Review Board (IRB) in carrying out the informed consent procedures.

- Signing the *NPAAS Consent*:
  - Once the participant's questions have been answered, ask her to sign and date two copies of the *NPAAS Consent* in the appropriate places. Sign two copies of the form yourself, as a WHI representative, and date the form. Give one copy of the *NPAAS Consent* to the participant and file the other copy in her file. Record on *Form 175 – NPAAS Visit 1* that the participant has signed the *NPAAS Consent*.
  - If a participant declines to sign the *NPAAS Consent*, do not continue further NPAAS activities. Record on *Form 175 – NPAAS Visit 1* that the participant did not sign the *NPAAS Consent*. Thank the participant and end the NPAAS visit. Change her status in WHIX task 174.

- Provide a WHI Folder: Provide each participant with a WHI folder to hold the NPAAS study materials provided at NPAAS Visit 1 (i.e., copy of *NPAAS Consent, Study At A Glance, Between Visits At A Glance, and Instructions for 24-hour Urine Collection*).

5.1.4.4 Provide *Study at a Glance* Handout

- Give participants, who consent to participate in NPAAS, a copy of *Study At A Glance* (copy available in *NPAAS Manual, Appendix C – NPAAS Participant Materials*). This handout provides a brief overview

of the activities that each participant will do as part of the Nutrition and Physical Activity Assessment Study.

5.1.4.5 Physical Measurements (height and weight)

- Collect the height and weight on the day of the NPAAS Visit 1. Use a measured height and weight (not an estimate). If possible, a stadiometer should be used to measure participant height. If a stadiometer is not available, then the preferred method would be to measure height from a stationary tape measure that is affixed to the wall. A measured height and weight are critical to correctly estimate total energy expenditure from the doubly labeled water and for the statistical modeling planned for the NPAAS study.

- Record the height and weight in centimeters and kilograms, respectively, on *Form 175 - NPAAS Visit 1*.

5.1.4.6 Spot Urine Collection – NPAAS Visit 1

5.1.4.6.1 General Information

- There are four spot urine samples collected at NPAAS Visit 1: a fasting sample (before the doubly labeled water [DLW] is administered) and three samples post DLW administration at approximately 2, 3, and 4 hours.

- Refer to *NPAAS Manual, Section 7.2.2.2.1 – Timing of the Spot Urine Collections* and *Section 7.2.2.2.2 – DLW Spot Urine Collection Steps* for information about the collection and processing of the spot urine samples at NPAAS Visit 1.

- **Thoroughly Rinse and Dry Collection Devices**
  The spot urine collections will be done using a urine collection hat (the hat is placed on the toilet, under the toilet seat). Each participant will have one urine collection hat for all her spot urine collections at NPAAS Visit 1. However, if more than one NPAAS participant is in the FC in a single day, do not share urine collection hats. The urine hat will need to be rinsed and thoroughly dried with a Bounty® paper towel between each spot urine. It is critical that the collection device be DRY before each urine collection because any water that contaminates the urine either during collection or processing will dilute the isotopic concentration of the DLW tracers. After each collection, the urine collection hat should be rinsed thoroughly and dried according to the following procedures:

  1. While wearing protective gloves, use a clean Bounty® paper towel to thoroughly dry the collection hat. Dispose of the used towel by placing it in a biohazard container.
  2. Obtain a second, clean Bounty® paper towel. Dry the collection hat a second time to remove additional droplets that may remain. Dispose of the second used towel by placing it in a biohazard container.
  3. Obtain a third, clean Bounty® paper towel. Dry the collection hat a third time to remove additional droplets that may remain. Dispose of the third used towel by placing it in a biohazard container.

- **Prevent Evaporation**
  Do not leave the urine open to the air so that it can either evaporate or exchange with the moisture of the air. A minute or two is okay, but do not leave the urine sitting around for 15 minutes without being capped. Ideally, have the participant's NPAAS specimen label on the cryovial; transfer the urine to the 5 mL cryovial, cap and store within 10 minutes. Refer to *NPAAS Manual, Section 7.2.2.2.1 – Timing of the Spot Urine Collections* and *Section 7.2.2.2.2 –DLW Spot Urine Collection Steps* for information about the collection and processing of the spot urine samples at NPAAS Visit 1.

5.1.4.6.2 Time 0 (Fasting) Urine Collection

- The Time 0 (fasting) spot urine needs to be collected, as soon as possible, after the participant's height and weight measurements have been taken. The fasting urine does not have to be the participant's first urine of the day BUT the urine must be collected BEFORE the participant drinks the DLW because the tracer gets in the urine very quickly (within 30 seconds). When the sample is collected, record staff ID (staff collecting urine) and time of collection on *Form 175- NPAAS Visit 1*.

- Use the procedures described in the *NPAAS Manual, Section 7.2.2.2.1 – Timing of the Spot Urine Collections* and *Section 7.2.2.2.2 –DLW Spot Urine Collection Steps* for information about the collection and processing of the spot urine samples at NPAAS Visit 1.

5.1.4.7 Administration of Doubly Labeled Water (DLW) – Fasting

5.1.4.7.1 DLW Doses

- Clinics will receive pre-filled bottles with doubly labeled water. The DLW doses are meant to provide enough of the tracer to the participant so that it can be measured easily, but not a huge dose, because the DLW is expensive. There are four dose sizes (A, B, C and D). These are designed for individuals who weigh respectively:
  A – Less than or equal to 65 kg
  B – 66 to 80 kg
  C – 81-105 kg
  D – Greater than or equal to 106 kg

5.1.4.7.2 DLW Administration

- Select the DLW bottle appropriate for the participant's weight. If a participant is right on the cusp between weights, staff can go either way (e.g., if the participant is 80kg, staff could give her either a B or a C bottle). If a scheduled participant should be getting a B bottle, but the clinic is out of B bottles, staff may use an A or a C bottle (one bottle size either direction is okay). Contact Lynn Fleckenstein (lflecken@whi.org) at the CCC if the FC is running out of a specific-sized bottle.

- Record 5 digit WHIX staff ID (staff administering DLW), the 7-digit dose weight of the bottle and the lot number of the bottle on *Form 175- NPAAS Visit 1*. The doses vary slightly, so it is critical to get the correct information recorded on the form.

- Either of the following procedures can be used for administering the DLW:

  Approach #1:
  1. Give the participant a straw (Straw #1) (without a flexible tip).
  2. Have participant use the straw (Straw #1) to drink all of DLW solution.
  3. Have participant remove Straw #1 from bottle and hold onto the straw.
  4. Add 50 mL of rinse water. Use the line provided on the DLW bottle to approximate this amount. Do not recap bottle.
  5. Mix well inside bottle 4. Participant re-inserts Straw #1 into bottle and drinks rinse water.

  Approach #2:
  1. Give the participant a straw (Straw #1) (without a flexible tip).
  2. Have participant use Straw #1 to drink all of DLW solution.
  3. Have participant push Straw #1 into bottle.
  4. Add 50 mL of rinse water. Use the line provided on the DLW bottle to approximate this amount. Recap the bottle.
  5. Mix rinse water well inside bottle and inside Straw #1.
  6. Remove cap.
  7. Give a new straw (Straw #2) to participant for drinking rinse water.

- If the participant would prefer to drink directly from the bottle, she may as long as her hands are steady enough to hold the bottle when she drinks.

- Record the time the participant begins drinking the DLW dose on *Form 175- NPAAS Visit 1*.

- Ask the participant to remain seated for about 15 minutes after drinking the DLW to minimize the small chance of dizziness that may occur upon drinking the loading dose.

5.1.4.7.3 Handling DLW Spillage

- Each FC will receive a supply of pre-weighed Kleenex tissues, each in a ziplock bag. The weight will be written on the bag.

- If there is a small spill where staff can mop it up (on the participant's cheek, on the floor, etc.), use the pre-weighed tissue to mop up the spilled DLW.

- Immediately return the tissue to the ziplock bag and seal it. Find the 'NPAAS DLW Spillage Container Visit 1' label on the participant's NPAAS label set.

- Place the participant's 'NPAAS DLW Spillage Container Visit 1' label on the bag and date the bag. Record 'Yes' for DLW spillage on the *Form 175-NPAAS Visit 1 (Qx.11.5)*. The same day, Fed-Ex (or similar courier with overnight, trackable service) the bagged tissue to Dr. Dale Schoeller at address below:

  Dr. Dale A. Schoeller
  Nutritional Sciences
  University of Wisconsin
  1415 Linden Drive
  Madison, WI 53706
  Phone #: 608-262-1082 FAX: 608-262-5860

5.1.4.7.4 Ordering and Storage of DLW

- When ordering an additional supply of DLW, FCs should place orders at least two weeks in advance of the date that the DLW is needed.
- The DLW bottles are prepared in a sterile manner and may be stored on a shelf at room temperature. The bottles do not need to be refrigerated. Keep them out of the sunlight, so that they don't get hot.
- While the bottles do not need to be refrigerated, the water tastes better chilled. The plastic bottles tend to give the water a bit of a 'plastic' taste, but chilling the water tends to make it a little more palatable. Suggestion: Chill the bottles overnight before giving them to an NPAAS participant to drink.

5.1.4.8 Complete Forms/Questionnaires and Present WHI Video, *Keeping Track of What You Eat*

All interviewer-administered and participant (self)-administered forms/questionnaires should be completed between other NPAAS Visit 1 activities, and should be planned to avoid interference with time-sensitive tasks (e.g specimen collections). Refer to *Figure 5.2 – Overview of NPAAS Visit 2* for the proper sequencing of form/questionnaire administration.

Please note that it is important to administer Form 35 (Personal Habits Update) and Form 45 (Current Supplements – Backup) sequentially, due to the use of similar time references. For example, both Forms 35 and 45 ask participants about *current* habits or supplement usage. **Forms 35 and 45 should be also be administered separately from (i.e. not in direct sequence with) Form 172 (PAR) or the AAFQ**, since these questionnaires refer to *past* (rather than *current*) activities.

- **Give the participant *Form 35 – Personal Habits Update* to complete.**
  - Information about the participant's smoking habits, alcohol use, and physical activity will be captured using *Form 35 (Personal Habits Update*).
  - Give the participant *Form 35 (Personal Habits Update*) to complete
  - Let the participant know that a staff person will review all forms with her before the end of her visit.
- **Give the participant *Form 60 – Food Frequency Questionnaire* to complete.**
  - Information about the participant's dietary intake will be captured using *Form 60 (FFQ)*.
  - Ask her to complete this form while she is waiting between urine collections. Remind the participant that a staff person will review all forms with her before the end of her visit.
  - Probe for additional information about missing answers. All adjustment questions (pages 2-4) and all summary questions (page 12) on the *FFQ* must be answered. Refer to Section 9 of the NPAAS manual for details about reviewing and editing the *FFQ*.
- **Complete *Form 45 – Dietary Supplement Use (Back-up).***
  - Information about the participant's current supplement use will be captured using *Form 45 (Dietary Supplement Use – Back-up)*.
  - The purpose of collecting an inventory of current supplements at NPAAS Visit 1 is to document the supplements a participant takes that could influence the levels of vitamins and minerals in her blood.
  - Remind the participant to report only current supplements taken at least once a week.
  - If the participant has forgotten to bring her supplements, arrange to have her bring them to NPAAS Visit 2 or call her at home to follow-up.
  - Record as '4- Non Routine in the "Visit Type" field
- **Give the participant *Form 171 – Viewpoints* to complete.**
  - Information about the participant's personal attitude, body image, and eating habits will be captured using *Form 171 (Viewpoints)*.
- **Give the participant the *Arizona Activity Frequency Questionnaire (AAFQ)* to complete or administer *Form 172 – Physical Activity Recall (PAR)* (depending upon random assignment).**
  Note: Participant will be asked to complete the other activity questionnaire at NPAAS Visit 2.
  - ***AAFQ*** - Information about the participant's daily activity, including occupational and non-occupational activities, will be captured using the Arizona Activity Frequency Questionnaire (AAFQ).
  - ***Form 172 (PAR)*** - Information about the participant's physical activity over the previous seven days. This form is administered by FC staff by interviewing the participant.
- **Have participant view WHI Video "Keeping Track of What You Eat."**

5.1.4.9 Provide Meal Replacement Beverage (MRB)

- One hour after administration of the doubly labeled water (DLW), provide the participant with an 8-ounce (240 mL) meal replacement beverage (e.g., Boost, Ensure, Sustacal etc) or other beverage of choice. Record "Yes" on *Form 175, Qx. 10.1 – MRB Consumed* and then record the amount (mL) and time the participant begins drinking the MRB (or other beverage). Encourage the participant to drink the beverage in a timely manner, rather than sipping.

5.1.4.10 Two Hour Urine Collection

- Two hours post DLW dose, have the participant provide a second spot urine sample. Use the procedures described in the *NPAAS Manual, Section 7.2.2.2.1 – Timing of the Spot Urine*

Collections and *Section 7.2.2.2.2 –DLW Spot Urine Collection Steps* for information about the collection and processing of the spot urine samples at NPAAS Visit 1.

- Recommended (but not required): Record staff ID (for staff collecting the urine) and time of collection on *Form 175 – NPAAS Visit 1*.

- If a participant has problems with the void, follow the procedures for handling collection problems, described in the *NPAAS Manual, Section 5.1.4.10.1 – Handling Collection Problems*.

5.1.4.10.1 Handling Collection Problems

- **Participant Produces No Urine**
  If a participant is unable to produce urine for the 2nd, 3rd or 4th spot urine collection, give her a little something to drink (i.e., ½ cup of water) and then have her try again in about half an hour. Record the amount and time of fluid intake on *Form 175 – NPAAS Visit 1,* (*Qx. 14 - Other Beverages Consumed*).
  - After a half-hour, if a participant is still unable to provide a spot urine sample, run warm water over the inner part of the participant's wrists. If still unable to provide a spot urine sample leave the appropriate urine collection question blank and indicate in the "Notes" section at the end of *Form 175-NPAAS Visit 1* that the participant was unable to provide a Time X urine specimen. Follow the procedures outlined in the *NPAAS Manual, Section 8.2.1 – Notes on NPAAS Forms* to notify Lynn Fleckenstein (206.667.2946) at the CCC.

- **Participant Produces Very Little Urine**
  If a participant produces very little urine for the 2nd, 3rd or 4th spot urine collection (less than 10 mL of urine), toss out the urine collection, give her a little something to drink and have her try again in about a half-hour. Record the amount and time of fluid intake on *Form 175 – NPAAS Visit 1, (Qx. 14 - Other Beverages Consumed*). If still unable to produce a urine sample, run warm water over the inner part of the participant's wrists. Note: Do not combine urine collections together to create a larger volume (i.e. combining first attempt with second attempt).

5.1.4.11 Three Hour Urine Collection

- Three hours post DLW dose, have the participant provide a third spot urine sample. Use the procedures described in the *NPAAS Manual, Section 7.2.2.2.1 – Timing of the Spot Urine Collections* and *Section 7.2.2.2.2 –DLW Spot Urine Collection Steps* for information about the collection and processing of the spot urine samples at NPAAS Visit 1.

- Record 5 digit WHIX staff ID (staff collecting the urine) and time of collection on *Form 175 - NPAAS Visit 1*.

- If a participant has problems with the void, follow the procedures for handling collection problems, described in the *NPAAS Manual, Section 5.1.4.10.1 – Handling Collection Problems*.

5.1.4.12 Three Hour Blood Draw (for women ≥ 60)

- For women 60 years of age and older, there is often an increase in the prevalence of post-void residual volume in the bladder. This means that the bladder does not totally empty out and this indirectly creates a problem in calculating energy expenditure. To help resolve this problem, a blood sample is used to check against the urine volume to detect if there was post-void residual volume.

- Three hours post DLW dose, draw a blood sample from women who are 60 years of age or older. The 3-hour blood draw must occur just after the 3-hour spot urine collection. If the 3-hour spot urine is slightly delayed, the 3-hour blood draw should also be delayed to immediately after that urine collection. Record the blood draw on *Form 175 - NPAAS Visit 1*. Use standard WHI blood handling and processing procedures described in the *NPAAS Manual, Section 7.2.2.2.6 – Time 3 Blood Draw (3 hours after DLW ingestion, for women 60 years of age or older)*.

- If a participant is under 60 years of age, place a note on the top of *Form 175 –NPAAS Visit 1* next to the participant's member ID label – "Participant is under 60". This will help NPAAS staff easily identify participants who do not need the 3-hour blood draw.

- If a participant has problems with the blood draw, follow the procedures for blood collection problems, described in the *NPAAS Manual, Section 7.4.4 – Blood Collection Problems*.

5.1.4.13 Collect and Review *Form 35, Form 60, Form 45, Form 171, and Form 172 or the AAFQ*

- *Form 35 (Personal Habits Update), Form 60 (FFQ), Form 45 (Dietary Supplement Use Back-up), Form 171 (Viewpoints), and either Form 172 or the Arizona Activity Frequency Questionnaire (AAFQ)* are to be completed and reviewed before the participant leaves the clinic.

- Make sure that there is an NPAAS participant bar code label in the designated space on the front page of each completed *Form 35 , Form 60, Form 45, Form 171, Form 172 and AAFQ* .

5.1.4.14 Four Hour Urine Collection

- Four hours post DLW dose, have the participant provide a fourth spot urine sample. Use the procedures described in the *NPAAS Manual, Section 7.2.2.2.1 – Timing of the Spot Urine Collections* and *Section 7.2.2.2.2 –DLW Spot Urine Collection Steps* for information about the collection and processing of the spot urine samples at NPAAS Visit 1.

- Record WHIX staff ID (staff collecting the urine) and time of collection on *Form 175 - NPAAS Visit 1*.

- If a participant has problems with the void, follow the procedures for handling collection problems, described in the *NPAAS Manual, Section 5.1.4.10.1 – Handling Collection Problems*.

5.1.4.15 Other Beverages

- It is important to record all the fluids that a participant drinks. Participants should not have free access to fluids – intake needs to be controlled. Record all fluid intake between the time of the DLW dose and the last spot urine sample at NPAAS Visit 1. Strongly encourage participants to drink fluids in a timely manner, rather than sipping.

- Participants should preferably have no more than 250 mL of fluids in addition to the 250 mL in the meal replacement beverage (MRB) for a total of 500 mL. But, it is important to get the urine specimens, so if a participant needs to go slightly above this 500 mL total to get the 3 or 4 - hour spot urine (e.g., the participant is dried out), staff may go ahead and provide a small amount of fluid. However, one liter is the upper limit; do not go above the one liter limit.

- Participant may have either caloric or non-caloric beverages, such as coffee, tea, or juice. These beverages do not interfere with the collection of the post-DLW protocol.

- When measuring the amount of other beverages, it is not necessary to measure the beverage in a graduated cylinder. A measurement within 10-20 mL of actual volume is close enough. Record the amount and time of beverage consumption on *Form 175 - NPAAS Visit 1 (Qx. 14 - Other Beverages Consumed*)

- If a participant does not drink any other beverages between hours 2-4 after the DLW, record '0' for *Qx. 14.1* (*Beverage 1 Amount*) and *Qx. 14.2* (*Beverage 1 Time*) on *Form 175 - NPAAS Visit 1*.

5.1.4.16 Explain and Provide Materials for 24-hour Urine Collection

- Near the end of NPAAS Visit 1, explain to the participant that she will need to collect all of her urine for 24 hours. She will begin her 24-hour urine collection on the day before her NPAAS Visit 2 (Day 14) and bring it back to her NPAAS Visit 2 appointment (Day 15). The 24-hour urine collection will be used to determine urinary nitrogen (a marker of protein intake).

- The 24-hour urine collection is preferably scheduled to coincide with the collection being completed for NPAAS Visit 2 on Day 15. However, for participant convenience, NPAAS Visit 2 may be scheduled for Day 14 or Day 16.

- Review the information provided on the *Instructions for 24-hour Urine Collection* with the participant (copy located in the *NPAAS Manual, Appendix C – NPAAS Participant Materials*). Provide clarification as needed.

- PABA

  The PABA procedure helps to determine completeness of the 24-hour urine collections. PABA is a type of B-vitamin that is absorbed but not metabolized by humans. A few participants may be allergic or hypersensitive to PABA and should not receive it.

  - Ask participants if they ever had hypersensitivity reactions to PABA-containing sunscreens or are allergic to PABA.
    - If yes (allergic to PABA):
      - Remove the PABA (B-vitamin) tablets from the urine collection kit and contact the CCC.
      - Complete questions 16.1 and 16.2 on *Form 175 – NPAAS Visit 1* (or *Form 177 – NPAAS Visit 3*) that the participant was not given the PABA (B-vitamin) tablets because she has had past hypersensitivity to PABA.
    - If no (**not** allergic to PABA):
      - Instruct participants to take one PABA (B-vitamin) tablet with each meal (or one after the first urine is flushed, one mid-day, and one in the evening). Point participants to the text on the *Instructions for 24-hour Urine Collection* where there is a reminder to "take one PABA (B-vitamin) tablet with each meal" on the day of the urine collection (use a Hi-Liter® marker to call attention to this text).
      - Instruct participants to refrain from taking acetaminophen (e.g., Tylenol®) or vitamins when collecting the 24-hour urine sample.
      - Ask participants to bring back the PABA tablet packaging with her to NPAAS Visit 2.
      - Complete questions 16.1 and 16.2 on *Form 175 – NPAAS Visit 1* (or *Form 177 – NPAAS Visit 3*)

- Provide the participant with a carrying bag containing the following 24-hour urine collection supplies:
  - *Instructions for 24-hour Urine Collection*, includes a *Record Sheet for 24-hour Urine Collection* (last page of instructions). There is a reminder to "take one PABA (B-vitamin) tablet with each meal" on the day of the urine collection (use a Hi-Liter® marker to call attention to this text).
  - 2 [3-liter] containers that have 2.0 gram (**0.5 level measuring teaspoon**) boric acid inside each bottle and are labeled with two stickers that read "Leave powder preservative inside bottle" and "Remember…take the PABA (B-vitamin) tablets". Remember to record the weight of each collection bottle per instructions on the *NPAAS 24-Hour Urine Collection Worksheet for Staff* before giving the collection bottles to the participant.
  - One urine collection hat
  - One plastic funnel
  - One large safety pin

- Two gel ice packs
- One plastic carrying bag
- 3 sealed PABA (B-vitamin) tablets.

- Inform the participant that a NPAAS staff person will call to remind her when to begin her 24-hour urine collection. Explain to the participant that she will need to bring her 24-hour urine collection, the *Record Sheet for 24-hour Urine Collection* and the gel ice packs to her NPAAS Visit 2. Also explain that she will also be asked to bring the PABA tablet packaging with her to NPAAS Visit 2.

- Give participant the Four Day Food Record booklet, portion size booklet, and measuring cups and spoons. Review the instructions at the beginning of the booklet with the participant. She should record on four non-consecutive days, including one week-end day. Ask if she has any questions. Remind her to return the completed booklet to visit 2.

5.1.4.17 Confirm Appointment for NPAAS Visit 2

- The NPAAS Visit 2 occurs on Day 15, fourteen days after NPAAS Visit 1.

- Confirm that the participant has a NPAAS Visit 2 appointment scheduled for a date that is two weeks after the first NPAAS visit. Schedule a NPAAS Visit 2 appointment, if necessary. If needed, NPAAS Visit 2 could be scheduled one day before or after Day 15.

- Give participant the *Between Visits At A Glance* sheet.

- Provide participant with the Four Day Food Record (4DFR), Portion Size Booklet, and measuring cups and spoons.

5.1.4.18 Provide Complementary Meal

- Thank the participant for her participation and provide a complementary meal (e.g., box lunch). The meal can be offered anytime after her last spot urine sample (i.e., 4-hour spot urine).

- Escort participant to exit.

5.1.4.19 Check Visit 1 Completeness

- Use the Completeness Checklist on the last page of *Form 175- NPAAS Visit 1* to check that all NPAAS Visit 1 activities have been completed. Complete any remaining tasks, if required. The staff member who reviews *Form 175 – NPAAS Visit 1* and checks completion of NPAAS Visit 1 tasks, designates completion by recording their 5 digit WHIX Staff ID on *Form 175- NPAAS Visit 1 (Qx. 18 – Visit 1 Completeness Staff ID*). Place all completed forms and questionnaires in participants NPAAS folder. All forms will be photocopied at the end of NPAAS Visit 2. Originals will be sent to the CCC and photocopies will be kept in the participant's NPAAS file at the FC (see *NPAAS Manual, Section 5.2.4., Post-NPAAS Visit 2 Activities*).

5.1.4.20. Urgent Visit Questions – Contacting the CCC

- FCs should direct on-the-spot visit questions in the following manner:
  Primary Contact: Lynn Fleckenstein – (206) 667-2946
  Alternate Contacts: Lesley Tinker – (206) 667-6894, Marian Neuhouser – (206) 667-4797

- For urgent situations during which the above NPAAS contact persons at CCC cannot be reached directly, FC staff should call the main CCC number (206-667-6883) and dial "0" to reach an operator. The operator can assist with contacting the NPAAS appropriate staff.

- FCs are advised to consider scheduling the first few NPAAS visits during hours when NPAAS staff at the CCC are likely be available for questions (i.e. no earlier that 7:30 a.m. PST).

5.1.5 Post-NPAAS Visit 1 Activities

5.1.5.1 Follow-up on Missing Forms

- FCs should have a data back-up plan if problems arise with collection of *Form 35 - Personal Habits Update, Form 45 – Dietary Supplement Use (Back-up), Form 60 – FFQ, Form 171 (Viewpoints), Form 172 (Physical Activity Recall)* and the *AAFQ.* If a participant leaves the clinic and NPAAS staff discover that any of these forms are missing or have incomplete data, assign an NPAAS staff person to follow-up with the participant within **1-2 days** of NPAAS Visit 1.

- Place all completed NPAAS Visit 1 forms in the participant's NPAAS file.

- **Note***:* The completed Visit 1 forms should not be photocopied or submitted to the CCC until the conclusion of Visit 2. Specific instructions for this procedure are provided in the NPAAS Manual, *Section 5.2.4.. – Post-NPAAS Visit 2 Activities.*

5.2 NPAAS Visit 2 (Day 15)

5.2.1 Overview of Activities

- *Figure 5.3 – Overview of NPAAS Visit 2 (Day 15)* provides an overview of NPAAS Visit 2.

Figure 5.3
Overview of NPAAS Visit 2 (Day 15)

Participant Arrival

- Greet participant
- Complete *NPAAS Visit 2 Participant Update Worksheet*
- Receive and process 24-hour urine collection and completed four day food record
  - Obtain weight
  - Obtain and process Time 0 (fasting) spot urine sample
  - Obtain and process Time 0 (fasting) blood draw
  - Conduct Indirect Calorimetry
  - Provide snack/meal
    - Obtain and process Time 1 spot urine (1-hour post fasting urine)
    - Participant completes AAFQ or interviewer administers PAR (*Form 172*)
- Provide Resting Energy Expenditure Results Letter
- Provide 24-Hour Recall Notification Letter
- Discuss repeat of NPAAS protocol in about 6 months. Complete *Consent for Future Contact and Continued Participation in this Study*
  - Schedule NPAAS Visit 3 appointment, if participant consents to repeat
- Begin necessary institutional paperwork for participant's $100 for time and travel
- Thank participant for completing the NPAAS protocol

- Complete and data-enter Form 174 – Participant Status Update

5.2.2 Pre-NPAAS Visit 2 Activities

5.2.2.1 Supplies and Forms

- NPAAS staff confirm that all supplies and forms needed for NPAAS Visit 2 are available for the participant's visit.

Supplies:

- NPAAS specimen label set (**must be** the remainder of the label set started at NPAAS Visit 1 for the specific participant)
- Royal blue and lavender (dry EDTA) blood collection tubes
- Corning cryovials with rubber O-Ring , 5mL (for spot urine aliquots) (2)
- 5.0 mL cryovials with blue color-coders (for 24 hour urine aliquots) (2)
- 2.0 mL cryovials (for blood and 24-hour urine aliquots) (9)
- Toploading digital scale to measure 24-hour urine collections
- Urine collection hat
- Disposable transfer pipettes
- 2.5 gallon plastic container (for mixing bottle contents of large urine collections)
- Snack
- Participant member ID labels
- Resting Energy Expenditure Results Letter
- 24-Hour Recall Notification Letter

Forms:

- *NPAAS Visit 2 Participant Update Worksheet*
- *Form 176 - NPAAS Visit 2*
- *NPAAS 24-Hour Urine Collection Worksheet for Staff*
- *Four Day Food Record Worksheet for Staff*
- *Arizona Activity Frequency Questionnaire* (AAFQ)
- *Form 172 (Seven-day Physical Activity Recall) and Script for PAR*
- *NPAAS Consent for Future Contact and Continued Participation in this Study*
- *Form 174 – NPAAS Status Update*

5.2.2.2 Pre-Visit 2 Reminder Call

- **Three days prior** to the participant's scheduled NPAAS Visit 2, call the participant. Remind her of the following:
  - Avoid consumption of vitamins (other than the study PABA pills) and acetaminophen-containing medications on the day *before* and the day *of* collection.
  - Start the 24-hour urine collection by collecting all of her urine beginning at 8 AM on the day before her Visit 2 using the specially provided urine collection kit.
  - take one PABA pill at each meal on the day of urine collection for all NPAAS participants excluding those who reported known allergies or hypersensitivities to PABA.
  - Fast for 12 hours prior to NPAAS Visit 2.

5.2.2.2.1 Review Instructions for 24-hour Urine Collection (Day 14)

- Review the steps the participant needs to take to complete her 24-hour urine collection. Ask her if she has any questions and answer her questions thoroughly. The following key points must be covered with the participant:
  - The collection period begins at 8:00 AM on the morning of the *DAY BEFORE* the participant's NPAAS Visit 2. The collection ends at 8:00 AM on the morning of the DAY OF her

appointment. The time can vary slightly as long as the collection time is 24 hours. If her appointment is scheduled early in the morning, she may start and end earlier than 8:00 AM.

- During the at-home collection, containers containing urine should be tightly capped and stored in a refrigerator on the bottom shelf at least 6 inches away from food. It may also be stored in a cooler with ice if preferred.
- During the urine collection period:
  1. The participant begins by emptying her bladder at 8:00 AM.
  2. She is to flush this urine. She will collect all urine ***after*** this point but record this time as "Start of collection" on the attached record sheet (*Record Sheet for 24-hour Urine Collection*).
  3. She should take one PABA (B-vitamin) tablet with each meal (or one after the first urine is flushed, one mid-day, and one in the evening). She is not to take acetaminophen (e.g., Tylenol®) or vitamins on the day before or the day of the collection period. If she has had hypersensitivity reactions to PABA in sunscreens, she should not have PABA (B-vitamin) tablets in her urine kit. Omit this instruction if participant is allergic or hypersensitive to PABA.
  4. She is to attach the large safety pin to her underclothing to serve as a reminder that she is collecting urine. It should be in a place that she can easily see when she goes to the restroom.
  5. The next time she goes to the restroom, she is to place the urine collection hat in the toilet (under the toilet seat) before urinating. It is important that she collects all of the urine. If she needs to have a bowel movement, she should collect the urine separately.
  6. After urinating, she is to use the plastic funnel to pour the urine from the urine collection hat into the collection container. Let the participant know that she should completely fill one urine bottle before starting to fill a second container.
  7. She is to continue collecting all urine for 24 hours (all day and night).
  8. At 8:00 AM (or 24 hours from her START time) the following day, the NPAAS participant is to empty her bladder into the urine collection hat and add this final urine to the collection container. The exact time of this last collection should be recorded on the "End of Collection" line on the *Record Sheet for 24-hour Urine Collection*.
  9. The participant is to note any problems with the urine collections on the *Record Sheet for 24-hour Urine Collection* (last page of *Instructions for 24-hour Urine Collection*). A copy of the instructions is available in the *NPAAS Manual, Appendix C – NPAAS Participant Materials*.
  10. The participant is to bring all urine collected, the *Record Sheet for 24-hour Urine Collection* and the gel ice packs with her to NPAAS Visit 2. In addition, she is to bring back the PABA tablet packaging with her to NPAAS Visit 2.

5.2.2.2.2 Confirm Appointment and Fasting Time

- Confirm the participant's NPAAS Visit 2 appointment. If the participant needs to re-schedule her clinic appointment (e.g., emergency situations), try to reschedule the appointment within ± one day. **Do not go longer than 20 days post NPAAS Visit 1.**
- Remind the participant to:
  - Refrain from eating any food for **12 hours** prior to her clinic appointment. Encourage the participant to drink water liberally during the fast; otherwise, she may become dehydrated which can be uncomfortable for the participant and result in difficulties collecting urine and blood samples. Black coffee or tea, without milk, cream or sugar (caffeinated or decaffeinated) is okay at NPAAS Visit 2 after fasting urine and indirect calorimetry have been completed.

- Take all her regular medications with water on the day of NPAAS Visit 2.
- Wear clothing which allows the sleeve to be easily raised above the elbow without constricting the blood flow to the forearm and hands and allows for ease of multiple urine collections.
- Possible Script: "You are scheduled for a clinic visit at (time and date). During this visit, you will undergo a test to measure the number of calories that you burn at rest. In order to obtain accurate test results, you must not eat after 8 PM the night before the visit or on the morning before the visit. You may not drink any calorie containing beverages on the morning of your visit. It is also important that you do not smoke or take any nicotine products (chew, nicotine gums or patches), any caffeine containing products (coffee, tea, or diet sodas, or drugs to stay awake) for 2 hours before the visit."

- Ask the participant if she has questions and provide information as needed.
- Remind her to bring her completed Four Day Food Record and 24-hour urine sample to Visit 2.

5.2.3 NPAAS Visit 2 Activities

5.2.3.1 Reception – Welcome

- When the participant first arrives for NPAAS Visit 2, have her check in at the reception desk. The Receptionist should:
  - Locate the participant's file.
  - Indicate a comfortable place where the participant may wait until she can be seen.
  - Notify the NPAAS Lead-Ops (or designee) that the participant is waiting.

5.2.3.2 Complete *NPAAS Visit 2 Participant Update Worksheet*

- Complete *NPAAS Visit 2 Participant Update Worksheet* to monitor and note potential changes that might influence the spot urine samples collected at NPAAS Visit 2. None of the questions on this worksheet will make a participant ineligible (i.e., travel, use of IV fluids, etc.). The NPAAS Lead-ops (or designee) reviews the *NPAAS Visit 2 Participant Update Worksheet* and takes the appropriate actions outlined below.
  - If a participant marks 'No' to questions 1-4 on the *NPAAS Visit 2 Participant Update Worksheet*, NPAAS staff should:
    - Record their 5 digit WHIX staff ID on *Form 176 – NPAAS Visit 2, Qx. 3 – Staff ID to Indicate Completion of the Visit 2 Participant Update Worksheet*.
    - Begin NPAAS Visit 2 activities.
  - If a participant marks 'Yes ' to question 1, 2 or 3 on the *NPAAS Visit 2 Participant Update Worksheet*, NPAAS staff should:
    - Note the participant's response on the line next to the appropriate question.
    - Record their staff ID on *Form 176 – NPAAS Visit 2, Qx. 3 – Staff ID to Indicate Completion of the Visit 2 Participant Update Worksheet*.
    - Begin NPAAS Visit 2 activities.
    - At the end of NPAAS Visit 2, follow the procedures outlined in the *NPAAS Manual, Section 8.2.1 – Notes on NPAAS Forms* to notify the CCC.
  - If a participant marks 'No 'to questions 1, 2 and 3, but 'Yes' to question 4 on the *NPAAS Visit 2 Participant Update Worksheet* (indicating that she has not fasted for 12 hours), NPAAS staff should:
    - Record their staff ID on *Form 176 – NPAAS Visit 2, Qx. 3 – Staff ID to Indicate Completion of the Visit 2 Participant Update Worksheet*.

- Refer to the procedures in the *NPAAS Manual, Section 5.2.3.7.1-Handling Non-Fasting Participants* and *Section 7.4.4.1 – Directions for Staff When Participants Are Not Fasted* to manage the blood draw for non-fasting participants.
- Begin NPAAS Visit 2 activities.

5.2.3.3 Receive 24-hour Urine Collection

- Follow procedures outlined in the *NPAAS Manual, Section 7.3.3.2.1 – Receive the 24-hour Urine Collection from Participant* to receive the participant's 24-hour urine collection, review her *Record Sheet for 24-hour Urine Collection*, and complete the relevant sections of the *NPAAS Visit 2 Form*.

Handling 24-hour Urine Collection Problems:

- If a participant arrives at the FC with an incomplete or contaminated 24-hour urine collection, the Lead-ops (or designee) be sure that problems are noted on the *NPAAS 24-hour Urine Collection Record Shee*t
- If a participant arrives at the FC without a 24-hour urine collection, the Lead-ops (or designee) decides how to handle the missing 24-hour urine collection using the scenarios provided below:
  - **Participant Forgot to Collect**: If a participant forgot to collect her 24-hour urine sample, reassign a day for the collection within **3 working** days. Continue with the remaining NPAAS Visit 2 activities.
  - **Participant Forgot Collection at Home:** If a participant completed her 24-hour urine collection but forgot the container(s) at home, ask her to return her 24-hour collection to the clinic as soon as possible, within **2 working** days. Continue with the remaining NPAAS Visit 2 activities.
  - **Participant Refuses to Collect 24-hour Urine**: If a participant refuses to complete her 24-hour urine collection, the NPAAS Lead-ops (or designee) continues with the remaining NPAAS Visit 2 activities and at the end of the visit, follows the procedures outlined in the *NPAAS Manual, Section 8.2.1 – Notes on NPAAS Forms* to notify the CCC.

5.2.3.4 Receive Four Day Food Record

- Review the Four Day Food Record using the *Four Day Food Record Worksheet for Staff and Four Day Food Forms Instructions*. Clarify entries as needed with participant.

5.2.3.5 Physical Measurements (weight)

- Measure the participant's weight in kilograms and record on *Form 176 - NPAAS Visit 2*. The same scale should be used at Visit 2 as was used for measuring the participant's weight at Visit 1.

5.2.3.6 Spot Urine Collection - NPAAS Visit 2

5.2.3.6.1 General Information

- There are two spot urine samples collected at NPAAS Visit 2: a fasting sample and a sample one hour later. Use the procedures described in the *NPAAS Manual, Section 7.2.2.2.1 – Timing of the Spot Urine Collections* and *Section 7.2.2.2.2 –DLW Spot Urine Collection Steps* for information about the collection and processing of the spot urine samples at NPAAS Visit 2.
- Use the guidelines provided in the *NPAAS Manual, Section 5.1.4.6.1 - General Information* to thoroughly dry collection devices and avoid leaving the urine sample open to evaporation.

5.2.3.6.2 Time 0 (Fasting) Urine Collection

- The Time 0 (fasting) spot urine sample needs to be collected, as soon as possible, after completing *NPAAS Visit 2 Participant Update Worksheet* and obtaining the participant's weight. Use the procedures described in the *NPAAS Manual, Section 7.2.2.2.1 – Timing of the Spot Urine Collections* and *Section 7.2.2.2.2 –DLW Spot Urine Collection Steps* for information about the collection and processing of the spot urine sample at NPAAS Visit 2.

- When the sample is collected, record staff ID (for staff collecting the urine) and time of collection on *Form 176 - NPAAS Visit 2*.

5.2.3.7 Fasting Blood

- A fasting blood sample is required for all participants at NPAAS Visit 2. Draw the participant's blood if she has been fasting for at least **12 hours**. Use standard blood handling and processing procedures described in the *NPAAS Manual, Section 7.3.3.2.3 – Time 0 (Fasting) Blood Draw*.

- Be sure to protect all the blood samples collected at NPAAS Visit 2 from natural and fluorescent light. For example, cover vacutainers with foil until processing is completed (refer to *NPAAS Manual, Section 7.3.3.2.3 – Time 0 (Fasting) Blood Draw*).

5.2.3.7.1 Handling Non-Fasting Participants

- For managing participants who arrive at NPAAS Visit 2 non-fasting, refer to *NPAAS Manual, Section 7.4.4.1 – Directions for Staff When Participants Are Not Fasted*.

5.2.3.8 Indirect Calorimetry

5.2.3.8.1 Overview of Indirect Calorimetry

- Indirect calorimetry is a measurement of resting energy expenditure. FCs will supply their own metabolic cart and train their own staff.

- Resting metabolic rate will be measured with a Deltatrac II Respiratory Gas Analyzer, a Sensormedics 29, or similar instrument. These are semi-portable units that measure the concentrations of oxygen and carbon dioxide in air streams entering and exiting a clear plastic hood placed over the participant's head. Oxygen consumption and carbon dioxide production are calculated from the change in concentration and flow rate. The measurement must be made under standard conditions and requires about 30-40 minutes to complete. A 30-minute rest period is required prior to the start of the indirect calorimetry test to ensure that resting energy expenditure is measured.

- The resting energy expenditure measurements will serve the following purposes:
  - Identify the physiologic determinants of total energy expenditure
  - Provide a measure of physical activity by difference when used in conjunction with total energy expenditure

5.2.3.8.2 Eligibility

- Participants who have self-reported claustrophobia, which would cause them to become anxious when the plastic RMR hood is placed over their head should be excluded from taking part in the indirect calorimetry procedures. The procedures require that the participant lie quietly for 30 minutes and anxiety caused by claustrophobia would produce inaccurate test results.

5.2.3.8.3 Indirect Calorimetry Procedures

- The indirect calorimetry measurement may be conducted either before or after the rest of NPAAS Visit 2 procedures, but the participant must remain fasted until the conclusion of the indirect calorimetry test and until the NPAAS Visit 2 fasting blood has been drawn.

- Below is an overview of the steps taken to measure energy expenditure (indirect calorimetry). Note most of these measures may be conducted by local GCRC staff, but whenever possible, we ask that this protocol be followed:

  1. Ensure that the equipment has been properly calibrated at the start of each day.
  2. Ensure that the equipment is turned on and warmed up for at least 30 minutes prior to using it with a participant.
  3. Ask the participant to lie down and rest quietly for about 30 minutes.
  4. If the participant feels cold, offer her a blanket. If the participant feels hot, alter the environment to insure that she does not sweat.
  5. Check that the monitor is in canopy mode. Change if needed (This may differ between instruments).
  6. Check that the monitor is in the artifact suppression mode with a 10 min start delay (This may differ between instruments).
  7. Check that the hoses from the hood to the metabolic monitor are connected and the unit is turned on.
  8. Perform a calibration of the metabolic monitor, as needed.
  9. After the initial 30 minute rest period, measure the resting metabolic rate as per instrument instructions.
  10. The printer should be reporting data on a minute by minute basis. If it is not printing, check the connections, printer power, or see the PRINTER SETUP.
  11. Proceed with the measurement for 30-40 min.
      - The technician must remain with the participant - monitoring gas flow alarms and visually checking for labored breathing to insure that gas flow does not fail.
      - The participant must remain at rest but not sleep.
      - The participant must not talk, except when necessary to communicate a potential problem. If the participant does talk, lift their arms to scratch an itch, shift their weight to prevent stiffness etc, indicate the time and movement on the printout using a pen or pencil.
      - Confirm that the participant is still thermally comfortable.
      - If the participant has to get up because she needs to use the bathroom, then the measurement can be terminated, but the participant will need to start over. The measurement sequence (i.e., steps 3-13) needs to be repeated beginning with a 10 min rest in place of the 30 min called for in the basic protocol.
  12. At 30 minutes, check the display data printout for a stable reading (steady state).
  13. End the measurement.
  14. Obtain the output data from the metabolic cart. Save the readings on a disk or other electronic format. If electronic output not available, FCs will enter output onto Excel spreadsheet (using the template provided by the CCC). .
  15. Remove the hood from over the participant's head.
  16. Ask the participant to sit upright.
  17. Help the participant to their feet and be sure that they steady. Remember that they have fasted and there is a small risk of hypoglycemia.
  18. Give the participant her snack, if she has completed the NPAAS Visit 2 fasting blood draw.

19. Transcribe the participant's resting energy expenditure results onto the Resting Energy Expenditure Results Letter and give it to the participant. Answer any questions, as needed.

20. Sanitize the canopy per local procedures.

- **Note:** The primary safety concern is that airflow through the hood is maintained while the hood is in place over the participant's head. Loss of flow due to a rare failure of the fan in the metabolic cart or due to a loose hose will cause discomfort and in an extreme case may cause asphyxiation. Although an alarm will sound if the unit does not detect breathing, the EE technician should remain with the participant throughout the measurement. Care should also be exercised when the participant stands-up after the measurement should dizziness develop secondary to the fast.

5.2.3.8.4 Post- Indirect Calorimetry Quality Check

- After completion of the indirect calorimetry test, complete a quality check on the following:
  1. The printout/electronic record is legible. If not, correct problem and reprint.
  2. The average RQ is between 0.75 and 0.9. Values outside of this range may indicate that the participant fasted longer than 15 h (<0.75), ate within the last 6 h (>0.93), or hyperventilated during the measurement (>0.93). Other possible explanations are very high fat diets (<0.75), a weight loss diet (<0.75) or very high carbohydrate diets (>0.93). If the participant admits to a recent meal, reschedule the test.
  3. Check that the coefficient of variation is less than 10%. Possible explanations are excessive participant movement, irregular breathing pattern, failure to suppress the first 10 min of the measurement, or instrument maintenance problems. If the first 10 min of the measurement were not deleted, manually calculate the average and SD without the first 10 min. If the revised coefficient of variation is less than 10%, record these values. If not, repeat the measurement of resting metabolic rate.

5.2.3.8.5 Indirect Calorimetry Data Management

- For data collection and management, FCs should use the following steps:
  - Obtain a printout of the participant's indirect calorimetry results (minute-by-minute and summary information). FC staff should also download the data from the metabolic cart's computer to a disk or other storage device.
  - FC staff should reformat the electronic data, using the template provided by the CCC. The printout serves as a backup and the (reformatted) electronic data serves as the primary data.
  - Place the participant's "NPAAS Indirect Calorimetry" label from her NPAAS label set on the first page of her indirect calorimetry printout.
  - Place the participant's Member ID on each page of her indirect calorimetry printout.
  - Photocopy a hard copy of the printout (minute-by-minute and summary information) and staple as a back-up.Place the xeroxed copy of the indirect calorimetry printout in the participant's NPAAS chart. This copy is a back-up. The CCC asks that all data be transmitted in electronic form to Lynn Fleckenstein at the CCC (lflecken@whi.org). The schedule for submitting this electronic data to the CCC should be consistent with the schedule for shipping participant visit forms (see *NPAAS Manual, Section 5.2.4.1 - Copying and Mailing Visit Forms for the CCC)*.

5.2.3.9 Snack/Meal

- After the fasting blood is drawn, give the participant a light snack (e.g., juice, fruit, or crackers) while she is sitting in a quiet, comfortable place, away from the blood-drawing area. Serve foods that will help to bring blood sugar back up quickly. Some examples include fruit juices, fruit, granola bars, crackers, English muffins, bagels or toast (offered with jelly, margarine or peanut butter).

5.2.3.10 One Hour Urine Collection

- A final spot urine sample is collected 1 hour after the Time 0 (fasting) sample. Use the procedures described in the *NPAAS Manual, Section 7.2.2.2.1 – Timing of the Spot Urine Collections* and *Section 7.2.2.2.2 –DLW Spot Urine Collection Steps* for information about the collection and processing of the spot urine sample at NPAAS Visit 2.

- When sample is collected, record staff ID (for staff collecting the urine) and time of collection on *Form 176- NPAAS Visit 2*.

- *Note that the Indirect Calorimetry may take 60 minutes, so plan the one-hour urine collection accordingly.*

5.2.3.11 Give the participant *Form 172 – Seven Day Physical Activity Recall (PAR)* to complete *or* administer the *Arizona Activity Frequency Questionnaire (AAFQ)* (depending upon random assignment)

- The NPAAS specimen label set will indicate which instrument should be administered at each of Visits 1 and 2. The participant should have completed the other activity questionnaire at NPAAS Visit 1.

- *Either Form 172 or the AAFQ* are to be completed and reviewed before the participant leaves the clinic. The form/questionnaire should be administered between other NPAAS Visit 1 activities, and should be planned to avoid interference with time-sensitive tasks (e.g specimen collections and indirect calorimetry).

- Make sure that there is an NPAAS participant bar code label in the designated space on the front page of *Form 172 or the AAFQ*.

5.2.3.12 24-hour dietary recalls

- Inform the participant that she will be telephoned by staff at the Fred Hutchinson Cancer Research Center for three 24-hour recalls. One recall will take place in the next few weeks, and then about once a month until three recalls are completed. The recalls are unannounced and she will be interviewed about all foods and beverages consumed the previous day.

- Give the participant the 24-Hour Recall Notification Letter.

- Ask her to please keep the portion size booklet she used when recording the four day food record – she will need to use it during the recall interviews.

5.2.3.13 Invite Repeat of NPAAS - (Reliability Subsample)

- After the final spot urine, give the participant the *Consent for Future Contact and Continued Participation in this Study*. Refer to *NPAAS Manual, Appendix B – NPAAS Consents* for a copy of the consent. Explain that this consent is asking the participant if she would be interested in participating in a repeat of the Nutrition and Physical Activity Assessment Study in about 6 months.

- Ensure that the participant understands that by checking the box "I am willing to participate in a repeat of the procedures in the WHI Nutrition and Physical Activity Assessment Study in about six months" – it means that she is consenting to be part of the reliability subsample (90 women).

- Explain that by agreeing to repeat the Nutrition and Physical Activity Assessment Study, she will be asked to repeat all of the study procedures she just finished (i.e., drinking doubly labeled water, providing blood and urine specimens (spot urines at the clinic visits and 24-hour urine collection at home), being weighed, and completing questionnaires). There should be at least six months between NPAAS Visit 2 and the first reliability subsample visit (NPAAS Visit 3).

- Note: Each FC strives to recruit about 20% of their participants for the reliability subsample (Chicago, Seattle, and Oakland will strive to recruit about 14 participants each. Tucson, Memphis, Worcester, New York, Madison, and Chapel Hill will strive to recruit about 8 participants each). This oversampling helps to ensure that there is an adequate number of participants available for the reliability subsample. Some participants recruited at the end of NPAAS Visit 2 may be unavailable in 6 months when the reliability subsample study begins. The CCC will monitor response rates for the reliability subsample and let FCs know if they need to recruit more or fewer participants for the reliability subsample.

5.2.3.14 Make Arrangements for Time and Travel Costs and Provide Thank You for Participation

- **Time and Travel Cost Arrangements**. When the participant has completed her NPAAS Visit 2 activities, initiate the paperwork required by the FCs local institution to process a check for the participant. The costs are included in each FC's total budget.

- **Thank You**. Thank the participant for her participation in the NPAAS study. If the participant has agreed to be part of the reliability subsample, let her know that NPAAS staff will contact her within the next 6 months to schedule NPAAS Visit 3.

5.2.4 Post-NPAAS Visit 2 Activities

5.2.4.1 Copying and Mailing Visit Forms for the CCC

- After Visit 2 has been completed, photocopy all visit forms for each participant. Place photocopies in the participant's NPAAS file. Place the originals in a manila envelope with the particpant's member ID label. Mail completed forms packets by federal express to the CCC every Friday for Monday delivery. Packets should be directed as follows:

Lynn Fleckenstein (NPAAS)
WHI Clinical Coordinating Center
Fred Hutchinson Cancer Research Center
1212 Aloha / M3-A410
Seattle, WA 98109

SECTION 6
NPAAS RELIABILITY SUBSAMPLE

6.0 Overview of NPAAS Reliability Subsample

- For a subset of 90 women (a 20% reliability sample), the NPAAS protocol is repeated approximately six months after her NPAAS Visit 2. The 90 women in the NPAAS Reliability Subsample are identified at the end of the NPAAS Visit 2 when they sign the *Consent for Future Contact and Continued Participation in this Study* indicating that they are willing to participate in a repeat of the procedures in the WHI Nutrition and Physical Activity Assessment Study. For additional information about the NPAAS Reliability Subsample, refer to the *NPAAS Manual, Section 5.2.3.13 – Invite Repeat of NPAAS – (Reliability Subsample)*.

6.1 Sample Size

- Each FC strives to have **20% of their** participants repeat the procedures for the NPAAS Reliability Subsample.
  - To ensure that FCs have the required number of participants available to repeat the procedures, each FC will recruit participants for the NPAAS Reliability Subsample as follows:

    Chicago, Seattle, and Oakland – 14 participants each

    Chapel Hill, Madison, Memphis, New York, Tucson, and Worcester – 8 participants each

6.2 Approaching Participants for the NPAAS Reliability Subsample

- Not all participants approached for the NPAAS Reliability Subsample repeat the procedures. The reasons a participant does not repeat the procedures include:
  - The participant is no longer available or interested.
  - The participant is no longer eligible (e.g., now using insulin or hypoglycemic agents).
  - The FC has already enrolled all the women needed for the NPAAS Reliability Subsample.
  - The participant was not able to complete all NPAAS Visit 1 and Visit 2 activities.
- When approaching participants for the NPAAS Reliability Subsample, keep the following in mind:
  - Approach and schedule participants for NPAAS Visit 3 in the same order they complete NPAAS Visit 2.
  - To start, limit the number of participants who complete NPAAS Visit 3 and Visit 4 to **14** participants (Chicago, Oakland, and Seattle) and **8** participants (the other six FCs). To accomplish this, consider the following:
    - Schedule all recruited participants, but let participants with the latest NPAAS Visit 3 dates know that they are on a waiting list and may not be needed.
    - Schedule participants on the waiting list so that their NPAAS Visit 3 date is at least five weeks after the last NPAAS Visit 4 has occurred for the first **10** participants (Chicago, Oakland, and Seattle) and the first 5 participants (all other FCs). This ensures that participants on the waiting list will not begin repeating the procedures until the FC is sure the participant is needed.
- After **14** participants (Chicago, Oakland, and Seattle) and **8** participants (all other FCs) have completed NPAAS Visit 3 and Visit 4, contact the participants on the waiting list. Thank these participants for their interest and willingness to participate and let them know that they are still on a waiting list.
- Women who meet the following criteria are eligible to participate in the NPAAS Relability Study:Completed NPAAS Visit 1 and Visit 2.
  - Do <u>not</u> take insulin or oral hypoglycemic agents to manage diabetes.*
  - Do <u>not</u> require supplemental oxygen.*

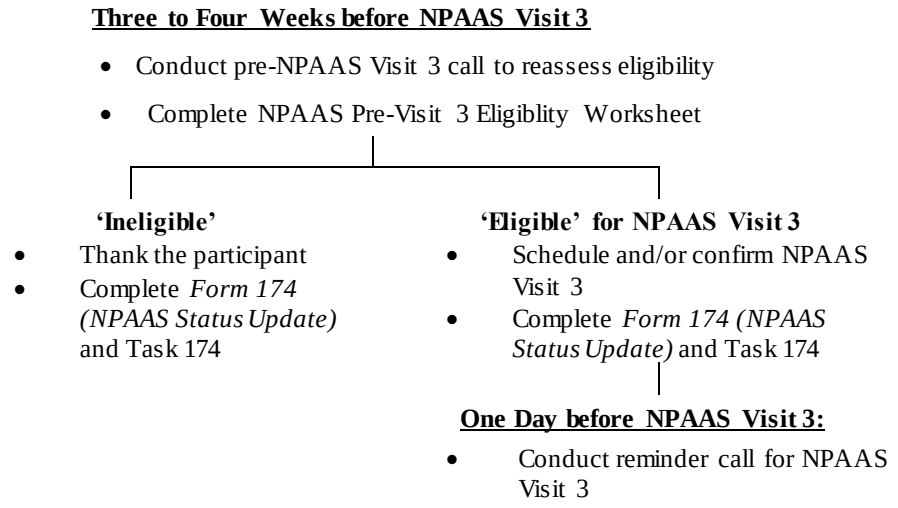
- Have not had blood transfusions, administration of blood products or administration of intravenous fluids in excess of 500mL in the week prior to the first clinic visit for this study or an expectation of the same during the period between visits 1 and 2 (including IV fluids administered as part of any administered anesthesia such as during a screening colonoscopy).*
- Will remain within 200 miles of their home during the week prior to the study and throughout the two week study period. **
- Have not lost or gained more than 15 pounds since NPAAS Visit 2. *
- Do not have bladder control problems that would make collection of a 24-hour urine specimen difficult.
- Do not have self-reported claustrophobia, which could cause the participant to become anxious when the plastic hood is placed over their head for the indirect calorimetry procedures. These procedures require that the participant lie quietly for at least 30 minutes, as anxiety caused by claustrophobia would produce inaccurate test results.

  * These factors interfere with the doubly labeled water measurements of energy expenditure, indirect calorimetry measures, or other study procedures.

  ****** Note: Travel of 200 miles or more (one-way) away from home or receipt of blood transfusions or IV fluids in the two weeks prior to NPAAS Visit 3 makes a participant temporarily ineligible. Ask the participant if she is willing to reschedule her NPAAS Visit 3 to accommodate the 2-week waiting period.

- Complete *NPAAS Pre-Visit 3 Eligibility Worksheet.* Refer to the *NPAAS Manual, Section 6.3.1 – Conduct NPAAS Pre-Visit 3 Reminder Call / Complete NPAAS Pre-Visit 3 Eligibility Worksheet.*
  - If a participant is ineligible:
    - Thank her for her time and interest in repeating the NPAAS Study.
  - If a participant is eligible:
    - Schedule and/or confirm her NPAAS Visit 3 appointment.
    - Ask the participant to refrain from traveling more than 200 miles away from home (one-way) or receiving blood transfusions or IV fluids in the 2 weeks prior to her NPAAS Visit 3 appointment.

Figure 6.1 - Reassessing Eligibility



6.3 Pre-NPAAS Visit 3 Activities

6.3.1 Supplies and Forms

- NPAAS staff confirms that all supplies and forms needed for NPAAS Visit 3 are available for the pre-Visit 3 eligibility reassessment call.

Supplies:

None

Forms & Print Materials:

- *NPAAS Pre-Visit 3 Eligibility Worksheet*
- *Form 174 – NPAAS Status Update*

6.3.2 Conduct NPAAS Pre-Visit 3 Eligibility Reassessment Call / Complete *NPAAS Pre-Visit 3 Eligibility Worksheet*

Figure 6.1 – Reassessing Eligibility provides an overview of NPAAS pre-Visit 3 activities

- The NPAAS staff completes the *NPAAS Pre-Visit 3 Eligibility Worksheet* for all participants receiving a pre-NPAAS Visit 3 call to reassess eligibility. **Note: This worksheet differs from the *NPAAS Visit 3 Eligibility Worksheet*, which will be completed in person when the participant arrives for NPAAS Visit 3.**

- Record the participant's eligibility summary on *NPAAS Pre-Visit 3 Eligibility Worksheet (Qx. 8 – NPAAS Pre-Visit 3 Eligibility Summary)*. The eligibility summary represents the participant's status at the end of the eligibility reassessment call:

  - 'Eligible': Participant is eligible to participate in a repeat of the NPAAS Study. Record 'eligible' if the participant meets the eligibility criteria, **regardless of whether or not she has accepted or declined participation in the Reliability Study.**

- If the participant is eligible, but is not interested in participating in the Reliability Study, then a 'declined' result should be indicated by marking "No" to question 8.1.1.
- If the participant is eligible and is interested in participating in the Reliability Study, then an "accepted" result should be indicated by marking "Yes" to question 8.1.1.

▪ 'Ineligible': Participant is not eligible to participate in a repeat of the NPAAS Study.

- Complete *Form 174 – NPAAS Status Update* only after the Pre-Visit 3 telephone call has been completed and the eligibility summary result is known. If a participant is unable to schedule her NPAAS Visit 3 appointment until she checks her family schedule, then a status result of "Pending scheduling" should be indicated on *Form 174* – NPAAS Status Update.

- Complete Task 174 within three days of the participant contact. File the completed *NPAAS Pre-Visit 3 Eligibility Worksheet* and *Form 174* in the participant's chart (or special NPAAS notebook). The *NPAAS Pre-Visit 3 Eligibility Worksheet* will copied and sent to the CCC after the conclusion of NPAAS Visit 4.

6.3.3 Pre-Visit 3 Reminder (one day prior to NPAAS Visit 3)

- NPAAS staff calls each participant one day prior to her NPAAS Visit 3. If a participant is scheduled for Monday, the call may be made on the preceding Friday. During the call, provide the same reminder information described in the *NPAAS Manual, Section 5.1.3.2 – Pre-Visit 1 Reminder Call*.

6.4 NPAAS Visit 3 (Day 1)

6.4.1 Supplies and Forms

- NPAAS staff confirms that all supplies and forms needed for NPAAS Visit 3 are available for the participant's visit. Refer to the *NPAAS Manual, Section 5.1.3.1 – Supplies and Forms*, for a list of the supplies to have available for NPAAS Visit 3.

Supplies:

None

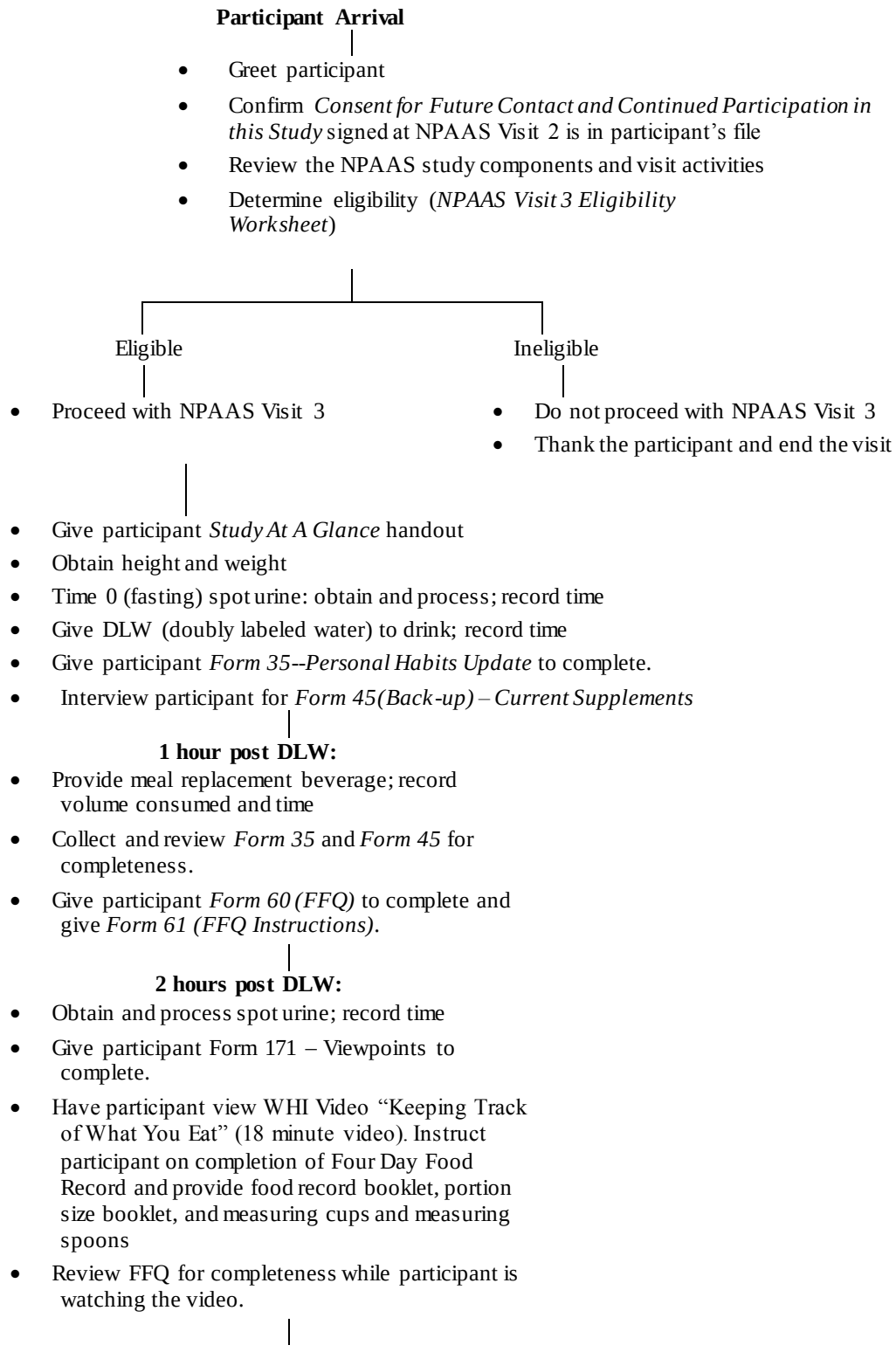
Forms & Print Materials:

- *NPAAS Visit 3 Eligibility Worksheet*
- *Consent for Future Contact and Continued Participation in this Study* (confirm that consent signed at NPAAS Visit 2 is in participant's chart)
- *Study At A Glance* Handout
- *Form 174 – NPAAS Status Update*
- *Form 177 – NPAAS Visit 3*
- *Form 60 – Food Frequency Questionnaire*
- *Form 61 – How to Fill Out the Food Questionnaire*
- *Form 35 – Personal Habits Update*
- *Form 45 – Current Supplements (Backup)* - (use hard copy; data entry will occur at CCC)
- *Arizona Activity Frequency Questionnaire* (AAFQ)
- *Form 171 - Viewpoints*
- *Form 172 (Seven-day Physical Activity Recall) and Script for PAR*
- *Four Day Food Record (Multiple Day Food Record)* & Portion Size Booklet (Serving Size Booklet)
- *Between Visits At A Glance* Sheet
- *NPAAS 24-Hour Urine Collection Worksheet for Staff*
- *24-Hour Urine Collection Kit*

6.4.2 Overview of NPAAS Visit 3 Activities

Figure 6.2 – Overview of NPAAS Visit 3 provides an overview of NPAAS Visit 3.

Figure 6.2
Overview of NPAAS Visit 3 (Day 1)



3 hours post DLW:

- Obtain and process spot urine; record time
- Obtain and process blood for women ≥ 60 years of age; record time
- Give participant AAFQ to complete or interview participant for *Form 172 (PAR)* (based on random assignment).
- Collect review all questionnaires **before participant leaves the clinic**

4 hours post DLW:

- Obtain and process spot urine; record time
- Provide participant with meal/snack
- Instruct participant on 24-hour urine collection and provide urine collection kit
- Review 4DFR instructions with the participant
- Give participant the *Between Visits At A Glance* sheet.
- Answer any questions about between visit tasks or indirect calorimetry to be completed at Visit 2.
- Schedule or confirm participant for NPAAS Visit 4 (15 days later)
- Thank participant and escort her to the exit.

- Complete blood and urine processing and storage; ensure correct labels are on each specimen
- Collate all NPAAS forms into participant's NPAAS folder for use at NPAAS Visit 2.
- Complete NPAAS Status Update (Task 174) in WHIX to indicate completion of Visit 3.

6.4.3 NPAAS Visit 3 Activities

- NPAAS Visit 3 activities are very similar to those described in the *NPAAS Manual, Section 5.1 – NPAAS Visit 1 (Day 1)*, with the exception of the following:
  - Staff confirms that the participant has a signed *Consent for Future Contact and Continued Participation in this Study* in her chart. A duplicate consent is not required.

- For a detailed description of NPAAS Visit 3 activities, refer to the *NPAAS Manual, Section 5.1.4 – NPAAS Visit 1 Activities*.

- Staff uses *Form 177 – NPAAS Visit 3* to record information collected at NPAAS Visit 3.

6.4.4 Post-NPAAS Visit 3 Activities

- For a detailed description of post-NPAAS Visit 3 activities, refer to the *NPAAS Manual, Section 5.1.5 – Post-NPAAS Visit 1 Activities*.

6.5 NPAAS Visit 4 (Day 15)

6.5.1 Pre-NPAAS Visit 4 Activities

6.5.1.1 Pre-Visit 4 Reminder Call

- NPAAS staff calls each participant two days prior to her NPAAS Visit 4. During the call, provide the same reminder information described in the *NPAAS Manual, Section 5.2.2.2 – Pre-Visit 2 Reminder Call*.

6.5.1.2. Supplies and Forms

- NPAAS staff confirms that all supplies and forms needed for NPAAS Visit 4 are available for the participant's visit.

Supplies:

- Refer to the *NPAAS Manual, Section 5.2.2.1 – Supplies and Forms,* for a list of the supplies to have available for NPAAS Visit 4.

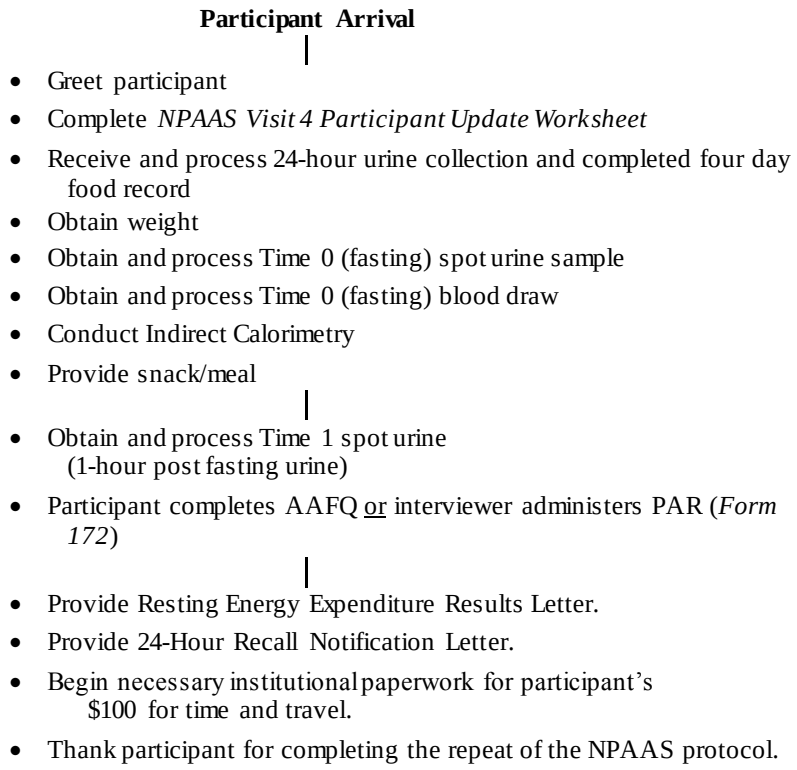
Forms and Print Materials:

- *NPAAS Visit 4 Participant Worksheet*
- *Form 178 - NPAAS Visit 4*
- *NPAAS 24-Hour Urine Collection Worksheet for Staff*
- *Four Day Food Record Worksheet for Staff*
- *Arizona Activity Frequency Questionnaire* (AAFQ)
- *Form 172 (Seven-day Physical Activity Recall) and Script for PAR*
- *Resting Energy Expenditure Results Letter*
- *24-Hour Recall Notification Letter*

6.5.2 Overview of NPAAS Visit 4 Activities

Figure 6.3 – Overview of NPAAS Visit 4 provides an overview of NPAAS Visit 4.

Figure 6.3
Overview of NPAAS Visit 4 (Day 15)



6.5.3 NPAAS Visit 4 Activities

- NPAAS Visit 4 activities are very similar to those described in the *NPAAS Manual, Section 5.2 – NPAAS Visit 2 (Day 15)*, with the exception of the following:
  - Staff do not invite the participant to return and do not collect the *Consent for Future Contact and Continued Participation in this Study* at the end of the visit.

- For detailed descriptions of NPAAS Visit 4 activities, refer to the *NPAAS Manual, Section 5.2.3 – NPAAS Visit 2 Activities* (omitting the invitation to repeat NPAAS).

- Staff use *Form 178 – NPAAS Visit 4* to record information collected at NPAAS Visit 4.

6.5.4 Post-NPAAS Visit 4 Activities

- For a detailed description of post-NPAAS Visit 4 activities, refer to the *NPAAS Manual, Section 5.2.4 – Post-NPAAS Visit 2 Activities*.

SECTION 7
BLOOD AND URINE COLLECTION, PROCESSING, AND SHIPMENT

7.0 Introduction

This section of the manual for the WHI Nutrition and Physical Activity Assessment Study (NPAAS) describes procedures for collecting, processing, and shipping blood and urine samples for the WHI NPAAS.

The NPAAS protocol calls for three types of specimen collection procedures:

1. Doubly labeled water (DLW) procedure of spot urine collections (and blood sample for women ≥ 60 years of age) for the analysis of energy expenditure.

2. 24-hour urine collection procedure for analysis of (a) urinary nitrogen (UN) as an estimate of protein intake and (b) select minerals as biomarkers of intake.

3. Fasting blood draw procedure for analysis of plasma phospholipids fatty acids and biomarkers of micronutrient intake, e.g., blood tocopherols, retinol, folate, carotenoids, B-vitamins, and selenium.

The three specimen collection procedures occur during two participant in-person clinic visits and one at-home 24-hour urine collection:

NPAAS Visit 1

During NPAAS Visit 1 (or NPAAS Visit 3), participants provide four spot urine collections for the DLW procedure and, for women ≥ 60 years of age, one non-fasting blood sample is taken three hours after ingesting the doubly labeled water. Participants receive instructions and a kit for the 24-hour urine collection procedure that is done one day before NPAAS Visit 2.

24-hour Urine Collection

One day before NPAAS Visit 2 (or NPAAS Visit 4), participants begin a 24-hour urine collection.

NPAAS Visit 2

During NPAAS Visit 2 (or NPAAS Visit 4), one fasting blood draw is taken and participants provide two spot urine collections for the DLW procedures. The participants bring in their 24-hour urine collection for processing.

Processing and shipping samples occurs as follows:

FC staff process and freeze samples from the blood and urine specimens in preparation for shipping.

FC staff ship specified serum, plasma, and urine samples to Fisher BioServices in Rockville, MD. Fisher BioServices ships the samples to the designated labs for analysis.

Laboratory quality control occurs as follows:

Five percent of biospecimen samples will have blinded duplicate aliquots collected for laboratory quality control (QC). Three FCs (Chicago, Oakland, and Seattle) will prepare all of the NPAAS QC samples.

7.1 General Guidelines

7.1.1 Safety Procedures - Precautions for Handling Blood

The NPAAS has adopted the Universal Precautions for blood collection, as was used with the WHI, processing and shipping. These precautions are intended as optimal "guidelines." These do not apply to urine unless there is visible blood in the urine. The term *Universal Precautions* refers to an approach to infectious disease control which assumes that every direct contact with body fluids is infectious. Also refer to the Occupational Safety and Health Administration (OSHA) guidelines which should be available from your institution for handling blood specimens.

Follow all local requirements for handling and disposing of blood and materials exposed to blood. Check with your institution's Health and Safety office for any additional requirements as there may be some local variation at each FC, which may be stricter or slightly less strict than those listed below.

The following are guidelines for all blood collection and processing procedures:

- Handle all blood specimens as potentially infectious material. Transmission of the infectious agents associated with hepatitis and acquired immunodeficiency syndrome (AIDS) via "needle stick" skin punctures have been documented.
- Wear disposable plastic latex gloves (or suitable substitute) when collecting and processing specimens. When drawing blood, change gloves between participants.
- Wear protective eye goggles, a face mask, a full face shield, or work behind a barrier shield at all times when processing blood. Regular glasses without splatter shields are not sufficient.
- Encourage staff having direct contact with blood specimens to get a Hepatitis B vaccination. Your local institution may provide Hepatitis B vaccinations for personnel handling blood specimens. The Hepatitis B vaccine provides immunity against Hepatitis B, an infectious, blood-borne disease causing inflammation of the liver with possibly serious or fatal consequences. The vaccination series involves three separate doses over six months. This protection is believed to last five to seven years at which time a booster dose may be needed.
- Wash your hands with soap and water:
    - Immediately after contact with blood or other infectious materials (even if you wore gloves).
    - Before and after using restroom facilities.
    - After you take off your gloves or other protective clothing.
    - Before and after each participant contact.
    - When you leave the work area where blood or other infectious materials are present.
- Do not use gloves if they are peeling, cracked or discolored, or if they have punctures, tears or other evidence of deterioration. Cover skin cuts or abrasions with a Band-Aid underneath the glove.
- Anyone performing a process where there is a potential for splashing of blood (e.g., the processing of blood) should wear a long-sleeved, buttoned-up, fluid-resistant lab coat.
- Use disposable lab coats, if available. Place disposable protective equipment in a labeled infectious waste container for disposal.
- Remove the disposable/non-disposable lab coat when leaving the blood draw area. The lab coat worn in the blood draw area should not be worn in other areas of the clinic.
- Tie back any long hair.

- If you accidentally sustain a contaminated (or used) needle stick, thoroughly cleanse the wound with soap and water. Notify the CC physician to order an analysis of the participant's serum for possible hepatitis or HIV antibodies. Complete a local accident report, as needed, and follow all appropriate OSHA guidelines as dictated by your institution.
- Do not manipulate needles, scalpel blades, or other contaminated sharp objects by hand. Do not bend, break or remove needles from disposable syringes by hand. Store unused needles in a secure cabinet when the CC is closed.
- Do not recap or re-sheath needles or sharp instruments unless absolutely necessary. If you need to recap a needle, cover it immediately after use. To recap the needle, use a cap holder designed for this purpose (placed in close proximity); or use a hemostat. Do not use your hand to recap the needle since this is the most common cause of sustaining a contaminated needle stick.
- Use 0.1% sodium hypochlorite (10% household bleach) to clean up any spills of blood, serum or urine. Use this solution on all work surfaces at the end of each day. Dilute one part household bleach with nine parts water to get a 0.1% sodium hypochlorite solution. Apply with a paper towel. Do not use an aerosol spray to apply the solution.
- Deposit all used needles, blood collection tubes, transfer pipettes and pipette tips in puncture-resistant containers for safe disposal.
- Place all other used blood processing supplies and blood products in biohazard bags for disposal. Also dispose of other materials exposed to blood in biohazard bags.
- Never perform any pipetting by mouth, especially if any blood or serum is involved.
- Avoid formation of potentially infectious aerosols by carefully removing stoppers from the vacutainer tubes and by careful pipetting and centrifugation.
- Never allow food or drink in the blood drawing or processing rooms. Do not store food or drink in the same refrigerator or freezer as blood samples.
- Do not eat, drink, apply cosmetics or lip balm, or handle contact lenses in work areas where blood or potentially infectious materials are present.
- Assume all laundry to be contaminated. Wear protective gloves when handling laundry. Bag laundry at the location where it was used. Double bag if soaking through is likely.
- Label all containers of infectious waste (i.e., biohazardous and medical waste), refrigerators and freezers containing blood or other potentially infectious materials with the biohazard symbol. The biohazard symbol must be black on an orange background.
- Transport all specimens or containers of blood and other potentially infectious materials in a secondary container (e.g., plastic bag or other container having a liquid-tight seal).

7.1.2 Safety Procedures - Precautions for Handling Urine

Follow relevant safety precautions described in the *NPAAS Manual Section 7.1.1 - Precautions for Handling Blood*. Additionally, follow institutional, local, and state guidelines and requirements for safe handling of urine as a biological specimen.

7.1.3 Training

FC staff person may draw, process and ship the blood and urine specimens for the NPAAS after completing the NPAAS training activities (refer to *NPAAS Manual Section 2.2.2 – Non-Lead NPAAS Staff*).

FC staff should also be familiar with local or state certification requirements. Keep a copy of any local and state requirements relating to the collection and processing of blood and urine products on file at the FC.

Because blood collection may involve a small amount of pain for the participant, it is important that phlebotomists review the techniques involved in the collection process. Phlebotomists should be highly experienced with vacutainer and butterfly blood collections, prepared to handle common problems such as fainting, and familiar with precautions to avoid exposing themselves to blood. Ideally they will be CPR certified. They should: 1) read "Collection and Handling of Laboratory Specimens: A Practical Guide" or similar phlebotomy manual; 2) wear clean white lab coats (with no blood stains) and appear generally neat and tidy; 3) wear name tags and introduce themselves to the participants before the blood draw; and 4) not chew gum or have any food items in their mouths during blood draws.

Because the study depends on the voluntary return of participants, the phlebotomist should make every effort to make the entire procedure as easy and painless as possible. The phlebotomist should remain calm and project an attitude of competence even when faced with the most nervous or inquiring participant. The best way to achieve this is to be thoroughly knowledgeable about all aspects of the procedures. This includes knowledge about the participant contact, the handling of each tube and the purpose of each sample.

7.1.4 Facilities

Phlebotomy Room

Perform blood draws in a private area such as an isolated room (blood drawing room) or in an area separated by room dividers. Equip the room with all the necessary blood drawing supplies. Use a counter or work table for all of the blood handling equipment and supplies. The blood drawing room should be clean and tidy with no obvious evidence of a previous blood draw such as used needles, blood stains, etc. A phlebotomy chair should be available for 15 to 20 minute periods to allow participants to be seated for a few minutes before the blood draw. Ideally, only the participant and phlebotomist should be in the room during the procedure.

Blood Processing Room

Equip the blood processing room with a refrigerated centrifuge, a small refrigerator and sufficient counter space for the processing of blood specimens. A -70°C freezer should be in or near the room. The room must have a sink and running water available. Use one counter area for processing the blood samples and another counter area for completing paperwork. Safety regulations state food and drink should not be kept in refrigerators or freezers in which you put the blood samples. Food should not be kept in the blood processing room even if it is not in the same refrigerator as the blood samples. Food should not be eaten in the blood processing room.

7.1.5 Supplies

Refer to *NPAAS Manual, Section 2.4.2 – Guidelines for Ordering Supplies* for information about supplies for blood and urine collection, processing and shipment.

7.1.6 Labels

7.1.6.1 Label Sets

The CCC provides the FCs with NPAAS label sets. **There is one NPAAS label set per participant**. This means that all the blood and urine specimens collected from a participant at NPAAS Visit 1 and

NPAAS Visit 2 have the same specimen identification number. Each label set contains 51 labels, all of which are needed for the NPAAS Visit 1 and NPAAS Visit 2 forms, sample tubes, and sample aliquots. Refer to *Figure 7.1 NPAAS Label Set (Visits 1 and 2)*.

- There are 21 labels for NPAAS Visit 1:
    - 10 labels with bar codes to be used for the forms, DLW spillage, and blood and urine aliquots.
    - 11 labels without bar codes used for blood tubes, as descriptors, or blank labels.
- There are 30 labels for NPAAS Visit 2:
    - 16 labels with bar codes to be used for the forms, indirect calorimetry, and blood and urine aliquots.
    - 14 labels without bar codes used as descriptors or blank labels.

NPAAS Visits 3 and 4 have a similar label set and are labeled appropriately with Visit 3 and Visit 4 text.

Place the participant's NPAAS label set in her chart. Write the participant's name on one of the blank labels in the NPAAS label set or place the participant's WHI member ID label on the back of the NPAAS label set so that it can easily be identified as belonging to that specific participant.

KEY POINTS (about the NPAAS label set):

- Each label set has an identification number.
- Label all the specimens collected at NPAAS Visits 1 and 2 with this identification number using the appropriate bar-coded NPAAS label.
- Label *Form 175 – NPAAS Visit 1* and *Form 176 – NPAAS Visit 2* with one label each There will be a check on key-entry of *Form 176 – NPAAS Visit 2* to ensure that that the label set identification number matches the one entered on *Form 175 – NPAAS Visit 1*.
- When time points are involved in specimen collection, those times are explicitly displayed on the label and the last digit of the aliquot matches the time point. For example, the NPAAS Visit 1 spot urine aliquots are as follows: 3**0**=Time 0, 3**2**=Time 2, 3**3**=Time 3, 3**4**=Time 4.
- The 2-digit aliquot identification numbers remain consistent across label sets for different participants. Only the label set identification number differs between participants.

The same key points about the NPAAS label set apply to NPAAS Visits 3 and 4.

Figure 7.1 – NPAAS Label Set (Visits 1 and 2) provides a sample label set for NPAAS Visits 1 and 2.

Figure 7.1 NPAAS Label Set (Visit 1 and 2)

**********	NPAAS Label Set 940001 X	**********
NPAAS VISIT 1 DO NOT USE	940001 X FORM 175 NPAAS Visit 1	940001 X AAFQ NPAAS Visit 1
	940001 X NPAAS DLW SPILLAGE Container Visit 1	
NPAAS Visit 1 Spot Urine DO NOT USE	940001-30 SPOT URINE Aliquot Visit 1 Time 0	940001-32 SPOT URINE Aliquot Visit 1 Time 2
	940001-33 SPOT URINE Aliquot Visit 1 Time 3	940001-34 SPOT URINE Aliquot Visit 1 Time 4
NPAAS Visit 1 60+ Blood DO NOT USE		940001 X NPAAS BLOOD Tube Visit 1
940001-40 Lavender Aliquot NPAAS Plasma Visit 1	940001-41 Lavender Aliquot NPAAS Plasma Visit 1	940001-42 Lavender Aliquot NPAAS Plasma Visit 1

Figure 7.1 NPAAS Label Set (Visit 1 and 2) continued

NPAAS VISIT 2 DO NOT USE	940001 X FORM 176 NPAAS Visit 2	940001 X PAR FORM 172 NPAAS Visit 2
	940001 X NPAAS Indirect Calorimetry	
NPAAS Visit 2 Spot Urine DO NOT USE	940001-50 SPOT URINE Aliquot Visit 2 Time 0	940001-51 SPOT URINE Aliquot Visit 2 Time 1
NPAAS Visit 2 Fasting Blood DO NOT USE	940001 X NPAAS BLOOD Tube Visit 2	940001 X NPAAS BLOOD Tube Visit 2
940001 X NPAAS BLOOD Tube Visit 2	940001 X NPAAS BLOOD Tube Visit 2	940001-02 Royal Blue Aliquot NPAAS Serum Visit 2
940001-03 Royal Blue Aliquot NPAAS Serum Visit 2	940001-04 Royal Blue Aliquot NPAAS Serum Visit 2	940001-05 Royal Blue Aliquot NPAAS Serum Visit 2
940001-10 Lavender Aliquot NPAAS Plasma Visit 2	940001-11 Lavender Aliquot NPAAS Plasma Visit 2	940001-12 Lavender Aliquot NPAAS Plasma Visit 2
NPAAS Visit 2 24-Hour Urine DO NOT USE	940001 X 24HR URINE Tube Visit 2	940001 X 24HR URINE Tube Visit 2

	940001-60 24HR URINE Aliquot 1.8 ML Visit 2	940001-61 24HR URINE Aliquot 1.8 ML Visit 2
	940001-70 24HR URINE Aliquot 4.0 ML Visit 2	940001-71 24HR URINE Aliquot 4.0 ML Visit 2

7.1.6.2 Placement of Specimen Lablels

Specimen labels that have been incorrectly placed on the cryovials may prevent Fisher Bioservices from being able to scan the barcodes. To ensure data quality, it is imperative that the barcode on the cryovial be scannable.

Please be sure that all staff know to place the specimen label on the cryovial as follows:

For the 2.0 mL cryovial:

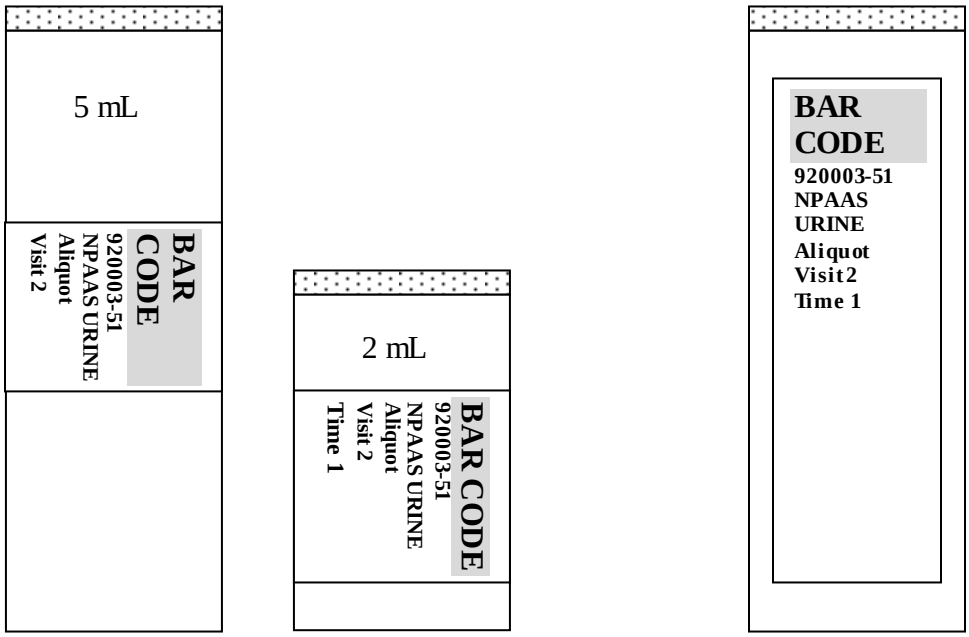
- Wrap the label around the circumference of the vial so that the short edge of the label is parallel with the long edge of the vial.
- Start with the non-barcode end of the label first so that when the label overlaps itself, the barcode is completely exposed.

For the 5.0 mL cryovial:

- Use the exact same procedure as outlined above for the 2.0 mL cryovial.
- IMPORTANT: Do not place the label on the 5.0 mL cryovial lengthwise (from end to end).

Please see *Figure 7.2 – Placement of Specimen Labels*.

Figure 7.2 Placement of Specimen Labels



CORRECT **INCORRECT**

7.1.7 Quality Control (QC) for Laboratory Analysis

Laboratory quality control will be assessed on a 5% subsample of NPAAS specimens, i.e., specimens from 24 participants NPAAS-wide for NPAAS Visits 1 and 2 and from 6 participants NPAAS-wide for NPAAS Visits 3 and 4. The analytic laboratories will receive the QC samples as blinded duplicate aliquots.

For efficiency, three FCs will prepare the NPAAS QC samples. Each of the three QC FCs will prepare samples from 8 participants at NPAAS Visits 1 and 2 and from 2 participants at NPAAS Visits 3 and 4. The QC participants need to remain the same for both NPAAS Visits (i.e., participants identified for QC at NPAAS Visit 1 will continue to be part of the QC at NPAAS Visit 2).

Blood QC.

- 3-hour post-DLW plasma (for women 60 years of age or older). For NPAAS Visit 1 (or NPAAS Visit 3), two of the three 3-hour post-DLW plasma aliquots will be labeled for QC.
- Fasting serum and plasma. For NPAAS Visit 2 (or NPAAS Visit 4), fasting serum and plasma samples collected from the WHI banked QC blood repository will be used for NPAAS QC. Staff do not need to prepare NPAAS QC serum or plasma aliquots for NPAAS Visit 2 (or NPAAS Visit 4).
- Participants will not need to have additional blood drawn for NPAAS QC at any of the NPAAS Visits.

Urine QC.

- DLW Spot Urine Collection. For each DLW spot urine collection at all NPAAS Visits, staff will prepare and label two additional 4 mL aliquots for QC.
- 24-hour Urine Collection. For the 24-hour urine collection processed at NPAAS Visit 2 (or NPAAS Visit 4), staff will prepare and label four additional 1.8 mL aliquots and four additional 4.0 mL aliquots for QC.
- Participants will not need to provide additional urine collections for NPAAS QC for either the DLW spot urine collections or the 24-hour urine collection.

For QC details related to each NPAAS Visit, refer to *NPAAS Manual Section 7.2.1.2. Quality Control (QC) Samples for NPAAS Visit 1 (or NPAAS Visit 3)* and *NPAAS Manual Section 7.3.1.2. Quality Control (QC) Samples for NPAAS Visit 2 (or NPAAS Visit 4) - QC FCs Only*.

7.2 NPAAS Visit 1 (or NPAAS Visit 3)

7.2.1 General Blood and Urine Sampling and Aliquot Schedule and Quality Control (QC)

7.2.1.1 Sampling and Aliquot Schedule

TIME	SAMPLE	ALIQUOT	QC (for the three QC FCs)
Time 0 (Fasting)	**Spot urine**	**One, 4 mL (5 mL cryovial)**	**Two, 4 mL (5 mL urine cryovial)**
Time 2 (Two hours post-DLW)	**Spot urine**	**One, 4 mL (5 mL cryovial)**	**Two, 4 mL (5 mL urine cryovial)**
Time 3 (Three hours post-DLW),	**Spot urine**	**One, 4 mL (5 mL cryovial)**	**Two, 4 mL (5 mL urine cryovial)**
Time 3 (Three hours post-DLW)	**Blood Draw Lavender dry EDTA, one 10 mL; for participants 60 years of age or older**	**Three 1.8 mL plasma (2 mL cryovial)**	**Label two of the three plasma aliquots with labels set aside for QC.**
Time 4 (Four hours post-DLW)	**Spot urine**	**One, 4 mL (5 mL cryovial)**	**Two, 4 mL (5 mL urine cryovial)**

7.2.1.2 Quality Control (QC) Samples for NPAAS Visit 1 (or NPAAS Visit 3) - QC FCs only

Refer to *NPAAS Manual, Section 7.1.7 Quality Control (QC) for Laboratory Analysis* for general blood and urine QC information.

The three QC FCs will collect QC aliquots by convenience sampling until the specified number of participants have had QC specimens collected. For a NPAAS QC participant at NPAAS Visit 1 (or NPAAS Visit 3), all urine QC aliquots must be from the same participant, and the same participant must have three plasma aliquots.

- For NPAAS Visit 1, each of the three QC FCs will collect QC aliquots from 8 participants (for a total of 24 participant-sets NPAAS-wide).
- For NPAAS Visit 3, each of the three QC FCs will collect QC aliquots from 2 participants (for a total of 6 participant-sets NPAAS-wide).

Labels. For each QC participant, use two additional NPAAS participant label sets for the NPAAS Visit forms and QC aliquots.

Blood. For each QC participant 60 years of age or older, label two of the three plasma aliquots at NPAAS Visit 1 (or NPAAS Visit 3) with labels reserved for QC.

Urine. For the spot urine collections at NPAAS Visit 1 (or NPAAS Visit 3), collect two additional 4 mL aliquots per NPAAS QC participant at each time point. Label one of these additional aliquots from one set of the two participant label sets reserved for QC and label the second additional aliquot from the remaining participant label set reserved for QC.

7.2.2 Day of NPAAS Visit 1 (or NPAAS Visit 3) (Day 1)

7.2.2.1 Before the Participant Arrives

1. Obtain a *Form 175 – NPAAS Visit 1* (or *Form 177 – NPAAS Visit 3*).
2. Obtain one NPAAS label set for the participant. (For QC FCs, obtain 3 label sets for each QC participant.)
3. Insert the *Form 175 – NPAAS Visit 1* and the NPAAS label set into the participant's file (or *Form 177 – NPAAS Visit 3* for the reliability subsample). Write the participant's name on one of the blank labels in the NPAAS label set or place the participant's WHI member ID label on the back of the NPAAS label set so that it can easily be identified as belonging to that specific participant.
4. Using the appropriate labels from the specimen label set, label the lavender dry EDTA tube and arrange in a test tube rack (for participants 60 years of age and older). Label and arrange the corresponding 2 mL cryovials in rack.
5. Label the 5 mL cryovials (with rubber O-ring seal for spot urine aliquots) and arrange in a rack.

7.2.2.2 During the Visit (Overview)

1. Verify that the participant is eligible and has signed the *NPAAS Consent* by reviewing *Form 175 – NPAAS Visit 1* (*or Form 177 – NPAAS Visit 3*), question numbers 3and 4.
2. Apply the NPAAS form label with the identification number on *Form 175 – NPAAS Visit 1* (*or Form 177 – NPAAS Visit 3*). For participants in the QC subsample, label the appropriate section of the NPAAS form.
3. Review with the participant the blood and urine collection procedures for NPAAS Visit 1 (or NPAAS Visit 3).
4. Collect the spot urine samples for Time 0 (*NPAAS Manual Section 7.2.2.2.3*), Time 2 (*NPAAS Manual, Section 7.2.2.2.4*), and Time 3 (*NPAAS Manual, Section 7.2.2.2.5*).
5. For participants 60 years of age or older, draw a blood sample 3 hours after DLW ingestion, in conjunction with the 3-hour spot urine collection (*NPAAS Manual, 7.2.2.2.6*).
6. Collect the spot urine sample for Time 4 (*NPAAS Manual, Section 7.2.2.2.7*).
7. Instruct the participant on the 24-hour urine collection and give her the 24-hour urine collection kit (*NPAAS Manual, Section 7.2.2.2.8*).

7.2.2.2.1 Timing of the Spot Urine Collections

The timing for each of the spot urine collections during the visit do not need to be precisely "on the hour," but the exact times do need to be recorded on *Form 175 – NPAAS Visit 1* (or *Form 177 – NPAAS Visit 3*). Subsequent collections may be collected at their scheduled times.

> Example 1: NPAAS Visit 1. A woman ingests the DLW at 9:00 AM. She is unable to produce a urine sample 2 hours after ingestion of the DLW (11:00 AM), but is able to 2-1/2 hours after (11:30 AM). On *Form175 – NPAAS Visit 1* (or *Form 177 – NPAAS Visit 3*), note 11:30 AM for the Time 2 Urine Collection. Obtain the Time 3 Urine Collection at 12:00 PM as scheduled.
>
> Example 2: NPAAS Visit 1. A woman ingests the DLW at 9:00 AM and she needs to void 90 minutes later (10:30 AM). On *Form 175 – NPAAS Visit 1* (or *Form 177 – NPAAS Visit 3*), note 10:30 AM for the Time 2 Urine Collection. Obtain the Time 3 Urine Collection at 12:00 PM as scheduled.

Coordinate the timing of the 3-hour urine and blood collections for women 60 years of age and older to occur together, i.e., if the urine collection needs be earlier or later than the scheduled Time 3 collection, collect the blood draw immediately after the urine collection.

7.2.2.2.2 DLW Spot Urine Collection Steps

At each time point when collecting the spot urine samples for the doubly labeled water analysis, follow these six steps:

1. Place the urine collection hat on the toilet (under the toilet seat) and ask participant to void into the hat. If she needs to have a bowel movement, she should remove the hat, have the bowel movement, and then replace the hat for the urine collection.
2. Complete the appropriate section of the NPAAS Visit forms.
3. Attach appropriate labels to cryovials. For participants in the QC subsample, label the extra cryovials.
4. Using a disposable transfer pipette, fill the labeled 5 mL Corning cryovial with 4 mL of urine specimen and store in freezer. Do not fill the cryovial to the top in order to allow for volume expansion upon freezing. For participants in the QC subsample, fill two additional labeled 5 mL Corning cryovials, each with 4 mL of urine specimen. Urine samples for the DLW analyses do not need to be centrifuged.
5. Discard remaining urine by flushing down the toilet.
6. Rinse and **dry** hat. **It is critical that the hat be thoroughly dried.**

Refer to *NPAAS Manual, Section 5.1.4.10.1 – Handling Collection Problems* for guidelines on handling problems with delayed or insufficient urine collection.

7.2.2.2.3 Time 0 (Fasting) Urine Collection

Before starting the visit procedures, verify that the participant is eligible and has signed the *NPAAS Consent* by reviewing *Form 175 – NPAAS Visit 1* (or *Form 177 – NPAAS Visit 3*), question numbers 3and 4.

Collect the **fasting spot urine** sample by following steps in the *NPAAS Manual, Section 7.2.2.2.2 - DLW Spot Urine Collection Steps*. Note the exact collection time on *Form 175 – NPAAS Visit 1* (or *Form 177 – NPAAS Visit 3*).

7.2.2.2.4 Time 2 Urine Collection (2 hours after DLW ingestion)

Collect the **2-hour spot urine** sample by following steps in the *NPAAS Manual, Section 7.2.2.2.2 - DLW Spot Urine Collection Steps*. The time does not need to be exactly at two hours; however, note the exact collection time on *Form 175 – NPAAS Visit 1* (or *Form 177 – NPAAS Visit 3*).

7.2.2.2.5 Time 3 Urine Collection (3 hours after DLW ingestion)

Collect the **3-hour spot urine** sample by following steps in the *NPAAS Manual, Section 7.2.2.2.2 - DLW Spot Urine Collection Steps*. The time does not need to be exactly at three hours; however, note the exact collection time on *Form 175 – NPAAS Visit 1* (or *Form 177 – NPAAS Visit 3*).

7.2.2.2.6 Time 3 Blood Draw (3 hours after DLW ingestion, *for women 60 years of age or older*)

Collect the **3-hour blood draw** for women 60 years of age or older, noting the exact time on *Form 175 – NPAAS Visit 1* (or *Form 177 – NPAAS Visit 3*). **Coordinate the time to occur just after the 3-hour spot urine collection**. Note the exact time on the NPAAS Visit form.

1. Follow general preparation for the participant (refer to *NPAAS Manual, Section 7.4.2 - Preparation for the Participants*)
2. Ensure the participant understands that you will draw 2 teaspoons of blood from her arm.
3. Following WHI venipuncture procedures (*NPAAS Manual, Section 7.4.3 - Venipuncture*), draw a single 10 mL lavender-dry EDTA tube 3 hours after the DLW ingestion. Attempt to draw a full tube. If the venipuncture is not successful or if you do not draw enough sample on the first occasion to fill the tube at least halfway, ask the participant for permission to attempt a blood draw a second time. If on the second attempt, less than 10 mL is collected, process the total amount collected. Refer to *NPAAS Manua,l, Section 7.4.4.7 - Deficient Serum or Plasma Samples*. Note: If the 3-hour spot urine collection is slightly delayed, then delay the 3-hour blood draw to immediately after that urine collection.
4. Note any missing collections in the "Notes" section of *Form 175 - NPAAS Visit 1*. Follow the procedures outlined in the *NPAAS Manual, Section 8.2.1 – Notes on NPAAS Forms* to notify the CCC.
5. Check the identifying information on the form and the labels to make sure the form is correctly labeled.

- The 3-hour post-DLW blood draw sample does not need to be protected from light.
- Bring the lavender tube to the blood processing area. Process the blood according to *NPAAS Manual, Section 7.5 - Blood Processing*.

7.2.2.2.7 Time 4 Urine Collection (4 hours after DLW ingestion)

Collect the **4-hour spot urine** sample by following steps in the *NPAAS Manual, Section 7.2.2.2.2 - DLW Spot Urine Collection Steps*. The time does not need to be exactly at four hours; however, note the exact collection time on *Form 175 – NPAAS Visit 1* (or *Form 177 – NPAAS Visit 3*).

7.2.2.2.8 Instructions for the 24-hour Urine Collection

General instructions for the 24-hour urine collection

- Refer to *NPAAS Manual, Section 5.1.4.16 – Explain and Provide Materials for 24-hour Urine* Collection for instructing participants how to conduct the 24-hour urine collection.

For participants who receive PABA (B-vitamin) tablets:

- Add boric acid powder to the urine collection bottles (boric acid supplied by the CCC).
  - Before giving the urine collection bottles to participants, add 2.0 gram of boric acid powder to each urine collection bottle. Measure the boric acid powder using a gram scale. If a gram scale is not available, add **0.5 level measuring teaspoon** of boric acid powder to each bottle*. Use standard safe laboratory practices by wearing a mask and gloves when adding the boric acid powder to the urine collection bottles. Boric acid is a weak acid, although it can be slightly irritating if inhaled.

    * The boric acid may be weighed or measured using household measuring spoons labeled, "for boric acid use only." When handling the boric acid, wear disposable gloves and preferably a disposable mask. Even though boric acid is a safe preservative, take precautions to avoid skin contact or inhalation.
- Place two stickers on each urine collection bottle: one sticker that reads "Leave powder inside" and a second sticker that reads "Remember…take the PABA (B-vitamin) tablets" (stickers supplied by the CCC).

- Apply a "Bottle #1" sticker to one of the collection bottles, and apply a "Bottle #2" sticker to the other bottle (stickers supplied by the CCC). Alternatively, a Sharpie® pen may be used to write "#1" on one collection bottle and " #2" on the other collection bottle.

- With a Sharpie® pen, write the participant's name on each bottle.

- Weigh each 24-hour urine collection bottle after adding the boric acid inside the bottle, after placing the three stickers on the outside of the bottle, and after labeling or writing on each bottle with the participant's name.
  - Follow the scale manufacturer's instructions for calibrating the scale.
  - Weigh each 24-hour urine collection bottle and transcribe the weight on the *NPAAS 24-Hour Urine Collection Worksheet for Staff. Transcribe the weights to the nearest 2grams. For example, 28 grams and 26 grams.*

For participants who do not receive PABA (B-vitamin) tablets:

- **Contact the CCC.**

7.3 NPAAS Visit 2 (or NPAAS Visit 4) (Day 15)

7.3.1 General Blood and Urine Sampling and Aliquot Schedule and Quality Control (NPAAS-modified)

7.3.1.1 Sampling and Aliquot Schedule

Sampling and Aliquot Schedule

TIME	SAMPLE	ALIQUOT	QC (for the three QC FCs)
Time 0 (Fasting)	**Spot urine**	**One, 4 mL (5 mL cryovial)**	**Two, 4 mL (5 mL cryovial)**
Time 0 (Fasting)	**Blood Royal Blue, three 7 mL COLLECT BEFORE LAVENDER DRY EDTA**	**Four 1.8 mL Serum (2 mL cryovial)**	**No NPAAS aliquots needed for QC. QC samples will be pulled from WHI banked QC samples.**
Time 0 (Fasting)	**Blood Lavender (dry EDTA), one 10 mL COLLECT AFTER ROYAL BLUE SERUM**	**Three 1.8 mL Plasma (2 mL cryovial)**	**No NPAAS aliquots needed for QC. QC samples will be pulled from WHI banked QC samples.**
Received from participant	**24-hour urine collection**	**Two, 1.8 mL (2 mL cryovial) and Two 4.0 mL (5.0 mL cryovial)**	**Four, 1.8 mL (2 mL cryovial) and Four, 4.0 mL (5.0 mL cryovial)**
Time 1 (One hour after Time 0)	**Spot urine**	**One, 4 mL (5 mL cryovial)**	**Two, 4 mL (5 mL cryovial)**

7.3.1.2 Quality Control (QC) Samples for NPAAS Visit 2 (or NPAAS Visit 4) - QC FCs only

Refer to *NPAAS Manual, Section 7.1.7 - Quality Control (QC) for Laboratory Analysis* for general blood and urine QC information.

The three QC FCs will collect QC aliquots by convenience sampling until the specified number of participants have had QC specimens collected. For an NPAAS QC participant at NPAAS Visit 2 (or NPAAS Visit 4), all urine QC aliquots must be from the same participant, and the same participant must have sufficient plasma for one sample aliquot and two QC aliquots.

- For NPAAS Visit 2, each of the three QC FCs will collect QC aliquots from 8 participants (for a total of 24 participants NPAAS-wide).
- For NPAAS Visit 4, each of the three QC FCs will collect QC aliquots from 2 participants (for a total of 6 participants NPAAS-wide).

Labels. For each QC participant, use two additional NPAAS participant label sets for the NPAAS Visit forms and QC aliquots. For NPAAS Visit 2 (or NPAAS Visit 4) carry forward use of the label sets stored with the participant chart from NPAAS Visit 1 (or NPAAS Visit 3).
Blood. Staff do not need to collect NPAAS QC serum or plasma aliquots at NPAAS Visit 2 (or NPAAS Visit 4). Quality control of blood sample analyses for NPAAS Visit 2 will be done using 30 blind duplicate pairs from banked WHI QC samples. For the 20% reliability subsample at NPAAS Visit 4, two pairs of banked WHI blind duplicate samples will be analyzed for QC.

Urine:

- For spot urine collections at NPAAS Visit 2 (or NPAAS Visit 4), collect two additional 4 mL aliquots per NPAAS QC participant at each time point. Label one of these additional aliquots from one set of the two participant label sets reserved for QC and label the second additional aliquot from the remaining participant label set reserved for QC.

- For the 24-hour urine collection, Visit 2 (or NPAAS Visit 4), centrifuge and collect four additional 1.8 mL aliquots and four additional 4 mL aliquots per NPAAS QC participant*. Label these additional aliquots from the participant's QC label sets.

  ***Note**: As indicated in the *NPAAS Manual, Section 7.3.3.2.5 – 24-Hour Urine Collection Processing*, QC sites will centrifuge additional urine from the 24-hour urine collection to fill the QC cryovials.

7.3.2 One Day Before NPAAS Visit 2 (or NPAAS Visit 4) (Day 14)

Refer to *NPAAS Manual, Section 5.2.2.2 - Pre-Visit 2 Reminder Call* and *Section 5.2.2.2.1. – Review Instructions for 24-hour Urine Collection (Day 14)*

7.3.3 Day of NPAAS Visit 2 (or NPAAS Visit 4) (Day 15)

7.3.3.1 Before the Participant Arrives

1. Obtain *Form 176 – NPAAS Visit 2* (or *Form 178 – NPAAS Visit 4*).
2. Apply the NPAAS form label with the identification number on the NPAAS Visit 2 form.
3. Insert the labeled *Form 176 – NPAAS Visit 2* and the label set into the participant's file (or *Form 178 – NPAAS Visit 4*).
4. Using the appropriate labels from the specimen label set, label the three blue serum tubes and one dry lavender EDTA tubes and arrange the tubes in a test tube rack.
5. Label the two 5 mL cryovial tubes (with rubber O-ring for spot urines collections)..
6. Label the two 5 mL cryovial tubes (with blue color-coder for 24-hour urine collections) and the two 2 mL cryovial tubes (for 24-hour urine collections).

7.3.3.2 During the Visit (Overview)

1. Verify that the *NPAAS Visit 2 Eligibility Worksheet* has been completed and that before the blood draw is taken that the participant has been fasting for twelve hours.
2. Review with the participant the blood and urine visit procedures for NPAAS Visit 2 (or NPAAS Visit 4).
3. Receive the 24-hour urine collection from participant (*NPAAS Manual, Section 7.3.3.2.1*).

4. Collect the Time 0 (fasting) spot urine sample (*NPAAS Manual, Section 7.3.3.2.2*).
5. Draw the Time 0 (fasting) blood sample (*NPAAS Manual, Section 7.3.3.2.3*).
6. Collect the Time 1 (1-hour) spot urine sample (*NPAAS Manual, Section 7.3.3.2.4*).
7. Process the 24-hour urine collection (*NPAAS Manual, Section 7.3.3.2.5*).

7.3.3.2.1 Receive the 24-hour Urine Collection From Participant

1. Verify that the participant's name on the bottle is the name of the participant. Place the collection container(s) in the refrigerator until after the Time 0 (Fasting) Urine Collection and Time 0 (Fasting) Blood Draw have been completed. Refer to *NPAAS Manual, Section 7.3.3.2.5 - 24-hour Urine Collection Processing* for the specific steps for processing the 24-hour urine collection.
2. Attach a participant member ID label to *Record Sheet for 24-hour Urine Collection* (last page - *Instructions for 24-hour Urine Collection*, given at NPAAS Visit 1*)*.
3. Review the *Record Sheet for 24-hour Urine Collection* with the participant to ensure all pertinent questions have been answered. The goal is to gather information about missed or contaminated urine collections.
4. The following responses on the participant's *Record Sheet for 24-hour Urine Collection* indicate the potential of a missed, spilled, or contaminated 24-hour urine collection:
   - 'Yes' response to Qx. 1: "Did you miss any urine collections?"
   - 'No' response to Qx. 4: "If you were away from home, were you able to collect your urine?"
   - 'No' response to Qx. 5: "Were you able to collect your urine during the night?"
   - 'Yes' response to Qx. 6: "Did you have any diarrhea during the collection period."
   - 'Yes' response to Qx. 7: "Did you spill any urine when pouring it into the bottle."
5. If the participant indicates that she missed any urine collections, use Qx. 2 – "If you missed urine collections, how many did you miss?" on the *Record Sheet for 24-hour Urine Collection* to note the total number of collections the participant missed.
6. For participants who have indicated a missed, spilled, or contaminated 24-hour urine collection, follow the procedures described in the *NPAAS Manual, Section 8.2.1 - Notes on NPAAS Forms* to notify the CCC.
7. Place the *Record Sheet for 24-hour Urine Collection* in the participant's NPAAS file.
8. Complete PABA-related questions on Form 176--*NPAAS Visit 2 (or* Form 178--*NPAAS Visit 4)*.

7.3.3.2.2 Time 0 (Fasting) Urine Collection

Collect the **fasting spot urine** sample following the steps outlined in *NPAAS Manual, Section 7.2.2.2.2 – DLW Spot Urine Collection Steps*. Note the exact time on *Form 176 – NPAAS Visit 2* (or *Form 178 – NPAAS Visit 4*).

7.3.3.2.3 Time 0 (Fasting) Blood Draw

Collect the **fasting blood draw**.

1. Verify that the participant has been fasting for twelve hours.
2. Follow general preparation for the participant (refer to *NPAAS Manual, Section 7.4.2. Preparation for the Participants*).
3. Ensure the participant understands that you will draw 2 Tablespoons of blood from her arm.

4. Follow the WHI venipuncture procedures (refer to *NPAAS Manual, Section 7.4.3. Venipuncture*).
   - Draw the royal blue tubes first, followed by the lavender tube.

     The reason that the royal blue tubes are drawn first is because they are preservative-free for trace element analysis. By drawing them first, the risk is lessened for contamination from the lavender EDTA tube.
   - Attempt to draw the full set of blood collection tubes. If you do not draw enough sample on the first occasion, follow the procedures in *NPAAS Manual, Section, 7.4.4.7 Deficient Serum and Plasma Samples*. The remainder of NPAAS Visit 2 may continue without the blood draw.
   - All of these samples must be protected from heat and light. Prepare aluminum sleeves for all of the tubes. Samples from these tubes will be analyzed for carotenoids, folate, and other nutrients which break down in white light. The aluminum foil cover will help protect the blood from light and prevent deterioration of the carotenoids.
5. Check the identifying information on the form and the labels to make sure that all are correctly labeled.

7.3.3.2.4 Time 1 Urine Collection (1 hour after Time 0 [fasting] urine collection)

Collect the **1-hour spot urine** sample following the steps outlined in *NPAAS Manual, Section 7.2.2.2.2 – DLW Spot Urine Collection Steps* The time does not need to be exactly at one hour; however, note the exact time on *Form 176 – NPAAS Visit 2* (or *Form 178 – NPAAS Visit 4*).

7.3.3.2.5 24-hour Urine Collection Processing

The 24-hour urine collection may be processed between other visit tasks, as time allows. The 24-hour urine collection is measured by weight by using a digital scale. Record weight in grams to the nearest 2 grams. Follow these steps for processing the 24-hour urine collection:

1. Weigh each urine collection bottle before removing any urine for processing. Be sure that the scale is calibrated according to the manufacturer's instructions. Transcribe the weight on the *NPAAS 24-Hour Urine Collection Worksheet for Staff*. Complete the *NPAAS 24-Hour Urine Collection Worksheet for Staff* to calculate the total weight of the 24-hour urine collection.
2. Document the total weight of the 24-hour urine collection on *Form 176 – NPAAS Visit, Qx. 8.1* (or *Form 178 – NPAAS Visit 4*, for the reliability subsample). Staff do not need to inspect the urine for color or degree of cloudiness. The lab will note any questionable samples.
3. Mix the 24-hour urine collection in its container thoroughly by inverting the tightly capped container ten times. If the participant brings in more than one 3-liter container, then mix the contents of the containers together using the steps outlined below:
   a. **If the total volume of the urine collection is less than 3 liters:**
      Use a plastic funnel to transfer all contents to one bottle. Cap tightly and invert ten times.
   b. **If the total volume of the urine collection is greater than 3 liters:**
      Use a plastic funnel to carefully transfer the contents from both collection bottles to a 2.5 gallon container. Cap tightly and invert ten times. Pour a portion of the mixed urine back into one of the 3 liter collection bottles for aliquotting.
4. Pour (or use a disposable transfer pipette) and centrifuge two 9 mL urine samples of the well mixed collection using the 10 mL centrifuge tubes provided for urine centrifugation. For QC FCs, pour and centrifuge four additional 9 mL urine samples. Centrifuge the 24-hour urine for 5 minutes at 1,300 xg. Refer to *NPAAS Manual*; Section 7.5.2 *Operating the Refrigerated Centrifuge* for instructions on how to obtain 1,300xg.
   - The intent of 24-hour urine centrifugation is to exclude particulates, but a small amount of sediment in the sample is acceptable.

5. Attach the appropriate labels to each cryovial.
6. Use a disposable transfer pipette to aliquot the centrifuged urine by placing:

   - 1.8 mL of urine into each of two labeled 2 mL cryovials.
   - 4.0 mL of urine into each of two labeled 5 mL cryovials.

   For QC FCs, prepare:

   - 1.8 mL of urine into each of four additional 2 mL cryovials.
   - 4.0 mL of urine into each of four additional 5 mL cryovials.

   Be careful not to overfill the cryovials because urine volume will increase upon freezing and the cryovials may crack.

7. Place labeled cryovials in the -70 degree Centigrade freezer.

7.4 Blood Draw Specifics

7.4.1 Preparation of the Blood Drawing and Processing Areas for All NPAAS Visits

Ensure the blood drawing and blood processing areas are equipped with the proper supplies.

7.4.2 Preparation for the Participants

NPAAS specifics:

- Each participant must have signed the *NPAAS Consent* authorizing the FC to draw blood before you can draw her blood.
- Ensure the participant understands the general aspects of blood collection as detailed in the *NPAAS Consent*.
- Verify with the participant that she has been fasting for 12 hours at NPAAS Visit 2. If needed, refer to *NPAAS Manual, Section 7.4.4.1 – Directions for Staff When Participants Not Fasted*.

Generalities

1. Blood drawing is standardized to the sitting position. Whenever possible, have the participant in the sitting position for five minutes immediately before venipuncture.
2. Review safety issues with the participant.

   - Ask the participant whether she bleeds or bruises easily. If she has had any problems with excessive bleeding or bruising at a venipuncture site, draw her blood only if approved by a Clinic Manager, FC physician or Principal Investigator (PI).
   - Ask the participant if she has ever been told she has a disorder related to blood clotting or coagulation or is taking any anticoagulants or aspirin. Continued bleeding may be a complication if the participant has any problems related to blood clotting or coagulation. To prevent bleeding and preserve the vein, you may need to apply pressure to the site for an extended period of time after you draw the blood. Stay with the participant to ensure the bleeding has stopped. Use Coban self-adherent wrap, or the equivalent, to keep in place for 10-15 minutes over the blood draw site.
   - Ask the participant if she has ever experienced fainting spells while having blood drawn. If she has experienced fainting spells during venipuncture, ask her the frequency of fainting spells. Proceed with the venipuncture if she has fainted only once before. If she advises that she frequently faints, consult the Clinic Manager, FC physician or PI before attempting the venipuncture. Provide

smelling salts, amyl nitrate, basin, or cold cloth, if needed. Have orange juice or fruit juice available to offer to the participant. It is advisable to have the participant lie down initially. Note the condition in the participant's file, and perform future blood draws while she is lying down. It is also advisable to loosen any tight clothing before drawing the blood, especially clothing around the neck. (See also *Section 7.4.4.6. Fainting*.)

7.4.3 Venipuncture

In general, refer to *WHI Manuals: Volume 2 – Procedures; Section 11.2.5 – Venipuncture*.

NPAAS specifics:

- Refer to *NPAAS Manual, Section 7.2 – NPAAS Visit 1 (or NPAAS Visit 3)* and *NPAAS Manual, Section 7.3 – NPAAS Visit 2 (or NPAAS Visit 4)* for the NPAAS sampling and aliquot schedule.
- Complete the remaining relevant sections of *Form 175 – NPAAS Visit 1* (for NPAAS Visit 1), *Form 176 – NPAAS Visit 2* (for NPAAS Visit 2), *Form 177 – NPAAS Visit 3* (for NPAAS Visit 3), or *Form 178 – NPAAS Visit 4* (for NPAAS Visit 4). **Note a missing blood draw in the "Notes" section of the NPAAS Visit form**. Follow the procedures outlined in the *NPAAS Manual, Section 8.2.1 – Notes on NPAAS Forms* to notify the CCC.
- Deliver the blood sample tube(s) and *Form 175 – NPAAS Visit 1* (for NPAAS Visit 1) or *Form 176 – NPAAS Visit 2* (for NPAAS Visit 2) to the blood processing area. Do the same for NPAAS Visits 3 and 4 for the reliability subsample, using *Form 177 – NPAAS Visit 3* or *Form 178 – NPAAS Visit 4*, respectively.

7.4.3.1 Wash Hands and Put on Gloves

Wash your hands with soap and water before every blood draw and put on pair of disposable plastic latex gloves.

7.4.3.2 Assemble the Vacutainer Holder

1. Attach the needle to the vacutainer holder.
2. Place the royal blue-stoppered tube in the vacutainer holder being careful not to break the vacuum

7.4.3.3 Identify Venipuncture Site

1. Position the participant's arm on the drawing table. Extend the arm toward you, palm up. Use a padded cushion under her elbow for comfort, if appropriate.
2. Wrap the tourniquet around the arm three to four inches (7.5 to 10.0 cm) above the venipuncture site.

   A tourniquet is used to increase venous filling. It makes the veins more prominent and easier to enter. PRECAUTIONS WHEN USING A TOURNIQUET: The tourniquet should be on the arm for the shortest time possible. **Never** leave the tourniquet on for longer than one (1) minute at a time. To do so may result in hemoconcentration or a variation in blood test values. (It was documented [Clinical Chemistry, 20:1513-1519, 1974] that changes in venous occlusion from 1 to 3 minutes led to a change in various blood components including increase in total lipids (4.7%) and cholesterol (5.1%) and decrease in other components.) If you must apply a tourniquet for the preliminary vein selection, release and reapply it after a wait of one minute. If the participant has a skin problem, put the tourniquet over the participant's shirt or use a piece of gauze or paper tissue so as not to pinch the skin.

3. Ask the participant to make a fist.
4. Identify a vein. Use the antecubital site of either arm as first choice. The median cubital vein is the one used most frequently. If the venipuncture of this vein is unsuccessful, use the cephalic and basilic veins as the next appropriate choices, followed by veins on the back of the hand. Palpate and trace the path of veins several times with the index or middle finger. Unlike veins, arteries pulsate, are more elastic and have a thick wall. Thrombosed veins lack resilience, feel cord-like and roll easily. If you cannot readily see superficial veins, ask the participant to open and close her fist. Lowering the lower arm over the arm of the chair will allow the veins to fill to capacity. Identify the best available vein.
5. Palpate the vein. If you do not feel a vein, try another arm or site (see *Section 7.4.4.4.- Difficult to identify venipuncture*).
6. Cleanse the vein site with the alcohol prep using a circular motion from the center to the periphery.
7. Allow the area to dry to prevent possible hemolysis of the specimen and a burning sensation to the participant when you perform the venipuncture.
8. If venipuncture becomes difficult, you may need to touch the vein again with your hand. If this happens, cleanse the site again with alcohol.

7.4.3.4. Perform Venipuncture

1. Explain the procedure to participant; for example, "I will be drawing a blood sample from your arm. You will probably feel a small prick when I insert the needle."
2. Grasp the participant's arm firmly, using your thumb to draw the skin taut. This anchors the vein. The thumb should be one or two inches (2.5 or 5.0 cm) below the venipuncture site.
3. Enter the vein in a smooth continuous motion with the needle bevel upward and parallel to the vein. Use a straight stab; do not poke around.
4. Make sure the participant's arm is in a flat and stable position.
5. Grasp the flange of the vacutainer holder and push the tube forward until the butt end of the needle punctures the stopper, exposing the full lumen of the needle.
6. Remove the tourniquet after blood is flowing into the tube. If no blood enters the tube, the needle may not be positioned in the vein. See *Section 7.4.4. Blood Collection Problems* for possible action.
7. Keep a constant, slight forward pressure (in the direction of the needle) on the end of the tube. This prevents release of the shutoff valve from stopping blood flow. Do not vary pressure or reintroduce pressure after completion of the draw.
8. Draw each vacutainer tube in order specified. Fill each tube as completely as possible, that is, until the vacuum is exhausted and the blood flow stops. If a vacutainer tube fills only partially, remove the vacutainer tube and attach another without removing the needle from the vein.
9. When the blood flow ceases, remove the tube from the holder. The shutoff valve re-covers the point, stopping blood flow until you insert the next tube (if necessary).
10. Draw the required blood tubes and place the royal blue-stoppered tubes into sleeves of aluminum foil to protect from the light.
11. Gently invert the lavender and light blue-stopped vacutainer tubes several times to ensure proper mixing of blood with the anticoagulants before placing the tubes in the rack. Do not invert the royal blue-stoppered tube.
12. Label the visit form and each tube with a blood sample label immediately after you complete the blood draw. (You can best do this while the participant is holding the gauze pad over the venipuncture site; see the steps below.)

7.4.3.5 Bandage the Arm

1. Under normal conditions:
   - To remove the needle, lightly place clean gauze over venipuncture site. Remove the needle quickly and immediately apply pressure to the site with a gauze pad. Discard needle with its cap into a needle box. If using a syringe with needle, dispose entire set-up into disposable container.
   - Have the participant hold the gauze pad firmly for one to two minutes to prevent a hematoma. Ask the participant to keep her arm extended. Bending her arm at the elbow increases the risk of developing a hematoma.
   - Apply an adhesive or gauze bandage over the venipuncture site after making sure that blood flow has stopped.
2. If the participant continues to bleed:
   - Apply pressure to the site with a gauze pad. Keep the arm elevated until the bleeding stops.
   - Wrap a gauze bandage tightly around the arm over the pad.
   - Tell the participant to leave the bandage on for at least 15 minutes.
3. Wash your hands.
4. Complete the remainder of the "Blood Collection" section of *the visit form.*
5. Deliver the blood sample tubes to the blood processing area.

7.4.4 Blood Collection Problems

7.4.4.1 Directions for Staff When Participants Are Not Fasted

For NPAAS Visit 2, do not draw the blood sample if the participant has not been fasting for at least 12 hours (water, black coffee or tea is OK). Note: A participant is considered to be non-fasting if she has consumed food, or caloric beverages within the 12 hours before the blood draw.

If she is not fasting and is:

- Willing to wait at the FC until the required number of hours of fasting has occurred, proceed with the blood draw at that time.
- Not willing to wait at the FC until the required number of hours of fasting has occurred, ask her if she is willing to reschedule her appointment.

If she is:

- Willing to reschedule - reschedule an appointment for the fasting blood draw and indirect calorimetry, and then proceed with the remaining NPAAS Visit 2 tasks. *Note: The Time 0 and Time 1 urine spot urine collection at NPAAS Visit 2* ***need not*** *be fasting.* Complete the appropriate section of the *NPAAS Visit 2 Participant Update Worksheet*.
- Not willing to reschedule - proceed with the remaining NPAAS Visit 2 tasks. Complete the appropriate section of the *NPAAS Visit 2 Participant Update Worksheet* and follow the procedures outlined in the *NPAAS Manual, Section 8.2.1 – Notes on NPAAS Forms* to notify the CCC.

7.4.4.2 Handling Participants Who Are Extremely Apprehensive About Having Blood Drawn

- Even though blood drawing is standardized for the sitting position, you may ask an extremely apprehensive participant to lie down, if there is a bed available in the FC.
- Do not force the participant to have blood drawn under any circumstances. It may help to explain to the participant that the blood drawing is designed to be as painless as possible. Sometimes it helps to let the participant go on with another part of the visit. It may also be helpful to have the participant relax in the blood drawing chair just so that you can check the veins in her arms, without actually drawing blood. If the participant has "good veins" you can reassuringly say, "Oh, you have good veins; there should be no problem." You may also consider using a butterfly needle to perform the draw if the participant is apprehensive. The butterfly needle is a thinner, smaller needle and may be less painful to the participant.
- Have the participant sit upright with jacket or sweater removed and with the sleeves rolled up to expose the antecubital fossa (elbow).
- Give the participant 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 is the blood collection process, the contact with the staff person who draws the blood, and the staff person's general attitude and competence.
- Reassure participants who are concerned about the volume of blood that the total amount of blood drawn is about two teaspoons at Visit 1 (for women who are 60 years of age or older) and about 2 tablespoons of blood at Visit 2. Assure the participants that they donate up to 10 times as much blood (450 ml) when they donate a pint of blood.

7.4.4.3 Special Consideration for Drawing Blood in the Elderly

The elderly pose some special blood drawing problems due to fragile and small veins, especially if the samples are drawn during a fasting period. The system of drawing blood by using vacuum tubes can increase this problem, causing veins to collapse due to high pressure exerted by the vacuum tubes. Steps outlined in *Section 7.4.4.4 Difficult Venipuncture* below may help to lessen some of the problems.

An important point in drawing blood is that the tourniquet must not occlude arterial flow; otherwise the problem of veins collapsing during the venipuncture may be accentuated. By using a syringe rather than a vacuum tube, you can control the pressure and reduce this problem. When using a smaller needle (for example, 22 gauge 1"), there is less chance of blowing (hematoma formation) a fragile vein.

7.4.4.4 Difficult-to-Identify Venipuncture Sites

1. Determine if the vein is difficult to identify, which may occur, if:
   - The palpitated vein feels small or rolls.
   - The participant has been stuck once already.
2. If the vein is difficult to find, check the back of the hand and forearm for venipuncture sites with larger veins. You can also try one or more of the following vein-dilation methods:
   - Be sure the room is not too cool.
   - Hot pack the venipuncture site with warm, wet towels for 3-5 minutes.
   - Have the participant wash her hands in warm water for 3-5 minutes.
   - Have the participant dangle her arm at her side with the tourniquet in place for one minute.
   - Use the blood pressure cuff as a tourniquet by pumping the pressure to 60-80 mm Hg.

3. If the vein is small, try a disposable syringe and 22-gauge needle or a butterfly initially.
4. Finish the venipuncture following the procedures outlined above.

7.4.4.5 Difficult Venipuncture

In general, refer to *WHI Manuals: Volume 2 – Procedures; Section 11.2.6.3 – Difficult Venipuncture*.

NPAAS specifics:

- The participants may be dehydrated having fasted for 12 hours (except water or black coffee or tea) in preparation for NPAAS Visit 2. Encourage the participants to drink water liberally during the fast. If this is a particular problem at your FC, consider offering the participants water as they arrive at the visit.
- If venipuncture for NPAAS Visit 1 (or NPAAS Visit 3) fails with the second attempt or second technician, do not attempt again. Proceed with the remaining tasks for NPAAS Visit 1 (or NPAAS Visit 3). **Note the missing blood draw in the "Notes" section of the NPAAS Visit form**. Follow the procedures outlined in the *NPAAS Manual, Section 8.2.1 – Notes on NPAAS Forms* to notify the CCC.
- If venipuncture for NPAAS Visit 2 (or NPAAS Visit 4) fails with the second attempt or second technician, ask the participant if she is willing to return at another time for a venipuncture. (Refer to *NPAAS Manual, Section 7.4.4.7 - Deficient Serum and Plasma Samples*). Then proceed with the remaining tasks for NPAAS Visit 2 (or NPAAS Visit 4).

Generalities

If you performed the venipuncture and a blood sample is not forthcoming, you may find the following manipulations helpful:

- If there is a sucking sound, turn the needle slightly or lift the holder to move the bevel away from the wall of the vein.
- If no blood appears, move the needle slightly in hopes of entering the vein. Do not probe. If unsuccessful, release the tourniquet and remove the needle. A second attempt can be made on the other arm.
- If the vein rolls, withdraw the needle slightly without coming back through the skin and try a second thrust.
- If the vein collapses, remove the vacutainer tube, call another staff person to reapply the tourniquet, ask the participant to open and close fist, and then reinsert tube. If still no blood appears in the tube, stop the procedure and use techniques in *Section7.4.4.4 Difficult- to-Identify Venipuncture Sites* above.

  If this is a problem on a particular participant, try using a butterfly needle rather than a vacutainer needle to draw the blood.

  The participants come to the FC visit fasting for 12 hours (nothing but water) and may be dehydrated. Encourage the participants to drink water liberally during the fast. If this is a particular problem at your FC, consider offering the participants water as they arrive at the visit.
- Loosen the tourniquet. You may have applied it too tightly, thereby stopping the blood flow. Reapply the tourniquet loosely. If the tourniquet is a Velcro type, quickly release and press back together. Be sure, however, that the tourniquet remains on for no longer than one minute at a time.
- Remove the tube and insert a second vacutainer tube. On rare occasions, the vacutainer tube may have lost its vacuum and thus may not draw blood into the tube even when the needle is positioned properly in the vein.

- If another technician is available, allow the other technician to attempt a venipuncture.
- If venipuncture fails with the second technician, request that the participant return at another time for a successful venipuncture. (See *Section 7.4.4.7 Deficient Serum and Plasma Sample*.)
- Reassure the participant that the inability to obtain a clean venipuncture is not any sign of a medical problem on her part.
- No single staff member should attempt more than two venipunctures on the same participant at any single visit.

7.4.4.6 Fainting

If participant shows signs of fainting (loss of color in the face, unusual sweating on forehead) or reports feeling dizzy:

- Finish drawing blood if possible but do not proceed if participant is clearly in trouble.
- Have participant lay her head on a table.
- Continue talking to participant to assess level of consciousness.
- Have participant lie down for 5-10 minutes after removing the needle; apply pressure on vein to prevent injuries from possible fall or seizure.
- Apply cool compress to her forehead.
- Have participant put a pillow or cushion under her knees.

If the participant faints:

- Withdraw the needle immediately.
- Apply pressure at the venipuncture site.
- Call for help.
- Apply a cool compress to her forehead.
- Use an ammonia capsule, if needed, by crushing the ampule and waving it under her nose for a few seconds.
- Once recovered, have the participant lie down on an exam table until she feels better.
- Take blood pressure readings to assess her recovery, if necessary.
- Offer the participant water, juice and food.

Note: If you do not collect a blood sample, reschedule the blood draw for another day.
Realize that the participant might be disoriented, embarrassed, or irritable and needs reassurance and attention. Recognize also that this incident may have an impact on future blood drawing and possibly on study adherence and must be handled well. Make a note of the difficulty in the participant's file for future reference.

7.4.4.7 Deficient Serum and Plasma Samples

Deficient blood from venipuncture:

- In general: If the venipuncture is not successful (no blood obtained) ask the participant for permission to attempt a second blood draw. Do not attempt a third time.
- Blue tube: If you do not get a full royal blue-stoppered vacutainer of blood (for serum), you should try to collect an additional half-tube immediately, with permission. After centrifuging the tubes, you can combine the serum from the half-tube with the serum from the first incompletely filled royal blue-stoppered tubes.

- Lavender tube: If you do not draw enough sample on the first occasion to fill the lavender tube at least halfway, attempt a blood draw a second time, with permission. If you have two partially filled lavender tubes, centrifuge each one and aliquot the plasma per procedures described in the *NPAAS Manual, Section 7.5.3.1 Preparation of Blood Cryovials*.

Deficient plasma or serum after centrifugation:

- Do not draw additional blood if plasma or serum is inadequate after centrifuging the blood samples.

7.5 Blood Processing

7.5.1 Blood Sample Handling and Processing: Timelimits

Procedures and time limits for blood processing are listed below. See also *Figure 7.3 - Guidelines for Blood Processing* .

1. Stand time:
   - Allow the royal blue-stoppered tubes to stand for at least 30 minutes at room temperature. The 30-minute waiting time is necessary to allow an adequate clot to form. If you frequently find fibrin clots in the centrifuged serum samples, try letting the samples sit for 45 minutes before centrifugation.
   - **Always protect blood samples for carotenoids (in royal blue-stoppered tubes) from natural and fluorescent white light. Store samples in covered containers or tubes.**
2. Refrigerate time:
   - Refrigerate the royal blue (after clotting) and 10 ml lavender tubes if it is not possible to centrifuge the samples within one hour after collection.
   - Set the samples in wet ice if a refrigerator is not available.
3. Centrifuge time:
   - Make every attempt to centrifuge the tubes between 30-45 minutes after collection. Set a timer, if necessary, as a reminder when the collected blood is ready for centrifugation.
   - Centrifuge the royal blue and light blue tubes within two hours of collection.
   - Centrifuge the 10 ml lavender tube within four hours of collection.
4. Aliquot time:
   - Aliquot the serum and plasma into the cryovials within 15 minutes after centrifugation.
5. Freeze time:
   - Freeze all aliquoted specimens within two hours of collection.

Figure 7.3
Guidelines for Blood Processing

Blood Collection Tube	Minimum Stand Time at Room Temp.	Maximum Stand Time at Room Temp. (before refrigeration)	Min. Time Post-blood Collection to Begin Centrifugation	Max. Time to Begin Centrifugation	Centrifuge Time	Post-Centrifugation Aliquot Time	Freeze Time Post Collection
Royal blue (7 ml)	30 min.	1 hr	≥ 30 min	2 hours post collection	10 minutes	15 minutes post-centrifugation	Within 2 hours post collection
Lavender (10 ml)	0 min	1 hr	Immediately post-collection	4 hours post-collection	10 minutes	15 minutes post centrifugation	Within 2 hours post-collection

7.5.2 Operating the Refrigerated Centrifuge

1. Set the centrifuge temperature at 4°C. (A setting in the 2° to 8°C range is acceptable.)
2. Load the rotor, being careful to place balanced tubes in buckets directly opposite to each other. If necessary, fill an empty tube with water, insert stopper and use as a balance for a blood sample tube. Be sure there is enough clearance for the tube and stopper when the buckets are in horizontal position.
3. Close the centrifuge cover and lock.
4. Centrifuge for ten minutes with brake off at a speed setting that will yield a relative centrifugal force (RCF) of 1,300 xg.

   You can obtain the desired RCF of 1,300 xg on various centrifuges by adjusting the revolutions per minute (RPM). You need to do this because the RPM setting of a centrifuge does not equal the RCF. The following equation shows the calculation of RCF:

   $$RCF = (1.118)(10^{-5})(r)(n^2)$$

   where r = rotating radius in centimeters (the distance from the center of the centrifuge rotor to the middle of the sample tube when the tubes in the swinging buckets are at the horizontal position) and n = revolutions per minute.

   Alternately, use *Figure 7.4 – Nomogram for Caluculating RCF* by lining up the radius in cm and RCF = 1,300 xg with a straight edge and reading the point at which the straight edge intersects the scale for RPM. For example, to achieve RCF of 1,300 xg with a centrifuge that has a radius of 10 cm, a straight line from these points on *Figure x.7.4* intersects the RPM scale at 3,400 RPM.
5. Note the time you started the centrifuge.
6. The centrifuge will stop automatically at the prescribed time. Allow the centrifuge to come to a complete stop before opening the cover. Do not use the brake to slow down the centrifuge. Using the brake may cause the RBCs to become resuspended in the plasma.
7. Remove the blood sample tubes one at a time and put in the test tube rack. Be careful not to tip the tubes and disturb the red cell layer. Re-centrifuge any tube containing red blood cells in the serum or plasma.
8. Consult the Centrifuge Service manual for other guidelines and for troubleshooting.

Equipment Quality Assurance

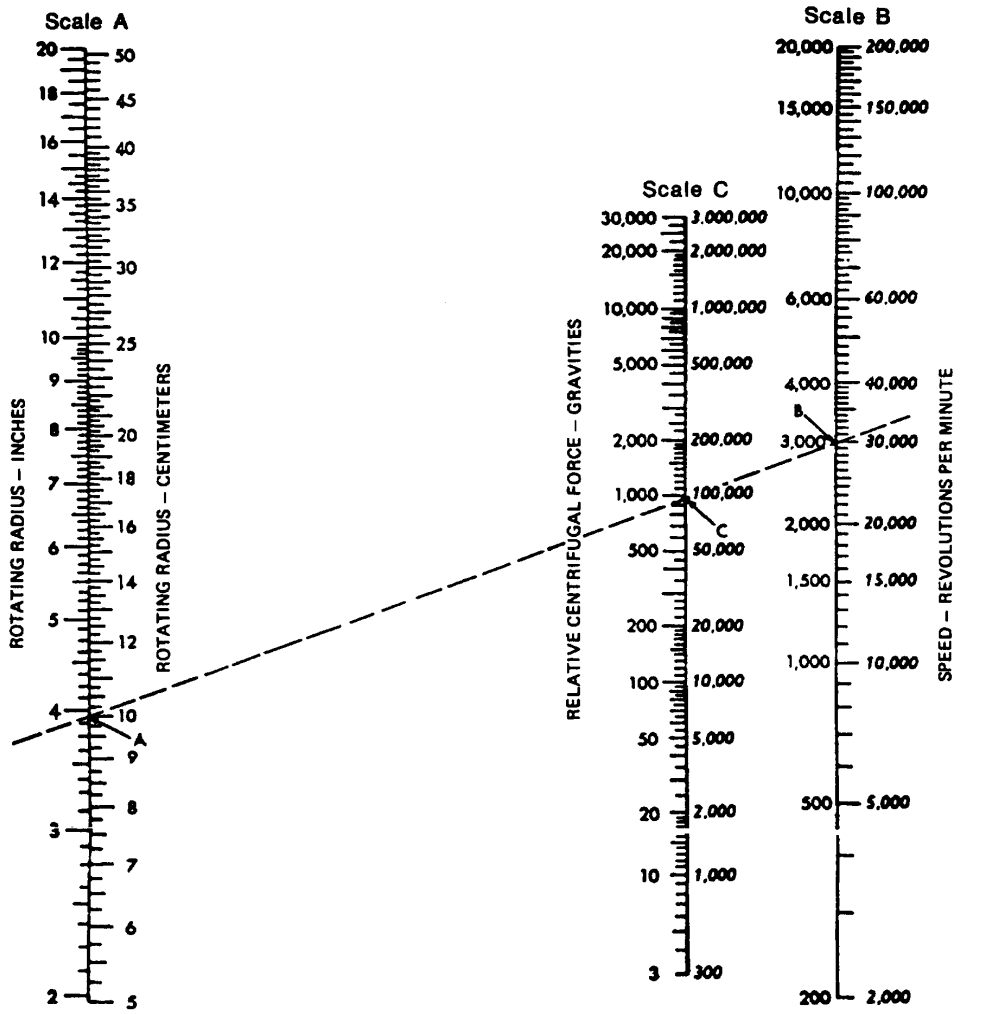
- **Centrifuge**

  Monitor the temperature of the refrigerated centrifuge daily using the temperature gauge on the centrifuge. Measure the temperature before centrifugation. Recording each temperature check on a log sheet is optional.

  Once each month, check the temperature of the centrifuge using a certified temperature thermometer in the 0°C to 10°C range. Do this even if you have an internal digital thermometer, as this will then also check the accuracy of the digital thermometer. You may do this by laying the thermometer on the bottom of the centrifuge.

  Once each year, check the speed of the centrifuge using a tachometer. Perform routine maintenance as suggested by the centrifuge manufacturer. A centrifuge maintenance log can be used to record these routine equipment checks and maintenance at the FC's discretion and is optional.

Figure 7.4 – Nomogram for Calculating RCF



*Modified from the IEC (a division of Damon Corporation, Needham Heights, Mass.) Relative Centrifugal Force Nomograph.

7.5.3 Processing Blood Samples

You can process several blood samples at a time as long as you follow the timing guidelines listed in *Section 7.5.1 Blood Sample Handling and Processing: Timelimits* and in *Figure 7.3 - Guidelines for Blood Processing*. To keep the blood samples you are processing separate, keep the blood collection and cryovials from each participant in a separate rack. This will help you aliquot the samples into the correct corresponding cryovials.

The following is an example of how you can set up your work area and process several blood samples at one time. You may change the procedure as needed to make the processing more efficient at your FC.

- Set up a separate rack for each participant's set of blood samples. Because the cryovials fit better in a rack with smaller holes, you can use two racks for each blood sample: the one with smaller holes for the cryovials in front, and one with larger holes for the blood collection tubes directly behind the smaller rack.

- When you first get the blood sample tubes, put the protected royal blue tubes in the appropriate racks to stand for 30 minutes and allow the blood to clot. Keep the tubes protected from light during this waiting time.
- Centrifuge the 10 ml lavender tube for ten minutes.
- While the samples are centrifuging, label the cryovials. Place the labeled cryovials in the smaller rack. Remove the caps and lay them in front of the corresponding cryovials in the rack.
- When the centrifuge stops, place the tubes from the centrifuge in the appropriate larger racks, placing the tubes directly behind the row of cryovials into which you will be placing the plasma.
- When 30 minutes have passed, centrifuge the royal blue tubes. While the tubes are centrifuging, you can complete processing of the lavender tube, if you have not already done so.
- Follow the procedure in *Section 7.5.3.1 Preparation of Blood Cryovials* for processing the serum from the royal blue tubes.

7.5.3.1 Preparation of Blood Cryovials

- Inspect each centrifuged tube for hemolysis. Re-centrifuge any specimen that appears to contain red cells suspended in the serum or plasma and then proceed with aliquotting, freezing and shipping. The analytic laboratory (ies) will make note of samples that may not be usable.
- Review *Form 175 – NPAAS Visit 1* (for NPAAS Visit 1) or *Form 176 – NPAAS Visit 2* (for NPAAS Visit Two) to determine the number of cryovials to prepare. Do the same for NPAAS Visits 3 and 4 with respective NPAAS Visit forms.

  When transferring the serum and plasma to the cryovials:

  Lavender-stoppered tube (NPAAS Visit 1 or NPAAS Visit 3; NPAAS Visit 2 or NPAAS Visit 4):

  - Remove the stopper from the Lavender-stoppered tube. **When you remove the stoppers from blood collection tubes that do not have hemoguards, remove them behind a work shield or wear a face shield to avoid potential exposure to the preservative.**
  - Transfer 1.8 mL of plasma into each of the three 2 mL cryovials. If you are not certain that you have sufficient plasma to fill each of the three cryovials with 1.8 mL plasma, first fill each vial with 1.0 mL plasma then add an additional 0.8 mL plasma to each vial until you run out of plasma. Screw the lids onto the three cryovials. Place the three vials in a freezer box dedicated to NPAAS and cover with a lid to protect the serum from white light. Insert the lavender stopper back into the blood collection tube. Place the blood collection tube and the pipette tip in a biohazard container.

  Royal Blue stoppered tubes (NPAAS Visit 2 or NPAAS Visit 4):

  - Remove the stopper from the royal blue-stoppered tubes.
  - Transfer 1.8 mL of serum into each of the four 2 mL cryovials. If you are not certain that you have sufficient serum to fill each of the four cryovials with 1.8 mL serum, first fill each of the vials with 1.0 mL serum. Then add an additional 0.8 mL serum to each vial until you run out of serum. This will help assure that you get sample in each of the four cryovials. Screw the lids onto the four cryovials. Place the four vials in a freezer box dedicated to NPAAS and cover with a lid to protect the serum from white light. Insert the royal blue stoppers back into the blood collection tubes. Place the blood collection tubes and the pipette tip in a biohazard container.

- Place the samples in the freezer box. Check that all cryovials are correctly labeled. Complete the *Blood Processing* portion of *Form 175--NPAAS Visit 1 (or Form 177—NPAAS Visit 3) or Form 176—NPAAS Visit 2 (of Form 178—NPAAS Visit 4)* as you place the aliquots into the freezer box, marking the corresponding aliquot on the form.

7.5.4. Freezing, Storing, Inventorying, Packaging, and Shipping Blood and Urine Aliquots

7.5.4.1. Freeze NPAAS aliquots at -70 degrees Celsius biological freezer.

Freeze NPAAS samples in a -70 degree Celsius biological freezer. If you do not have a -70°C freezer immediately available, put the cryovials in a -20°C freezer immediately after aliquoting. Then transfer to a -70°C freezer as soon as possible but no longer than two days (over the weekend). Placing the samples on wet ice or dry ice does not sufficiently preserve the sample, so you must put the samples in at least a -20°C freezer. Do not thaw the samples after freezing. If frozen samples are thawed (i.e., the result of a freezer failure) contact the CCC for instructions.

Equipment Quality Assurance

- Freezer

  Work with the person at your institution who is responsible for freezers to be sure that biological freezer temperatures are monitored daily. Use a certified low temperature thermometer in the -70°C range or a digit temperature display that may be on the freezer. If you use the digit display for the daily temperature checks, check the temperature of the freezer using a certified thermometer in the -80°C to-60°C range at least once each month. Recording each temperature check on a log sheet is optional.

  Each month check the CO_2 tank to be sure it has not been emptied. Also test the alarm system to be sure it will sound should the freezer temperature rise above -50°C. As of January 1997, FCs are required to have an alarm system attached to each -70°C freezer. An appropriate system must have the capacity to monitor the freezer and to call multiple phone numbers should malfunction occur. Perform routine maintenance as suggested by the freezer manufacturer such as routine freezer defrosting and record in freezer maintenance log. Make sure there are no samples in the freezer when you defrost it. You can coordinate the timing of the defrosting with the shipment of samples to Fisher BioServices; or use temporary storage in another freezer during the defrosting.

7.5.4.2. Store NPAAS aliquots in boxes dedicated to NPAAS.

- For the 2 mL cryovials: Use the same type of boxes as for WHI. Blood and urine aliquots may be in the same box.
- For the 5 mL DLW urine cryovials: Use the boxes ordered for NPAAS. Do not store 1.8 mL cryovials in the boxes designated for the 5 mL aliquots.
- Using a black Sharpie® marker, mark NPAAS in large letters on the side of the top of the box and the side of the bottom of each box.
- Store aliquots in a way that facilitates shipping all samples from NPAAS Visit 1 and NPAAS Visit 2 together for a given participant (and similarly for NPAAS Visit 3 and NPAAS Visit 4 for the reliability subsample).

7.5.4.3. Inventory stored aliquots.

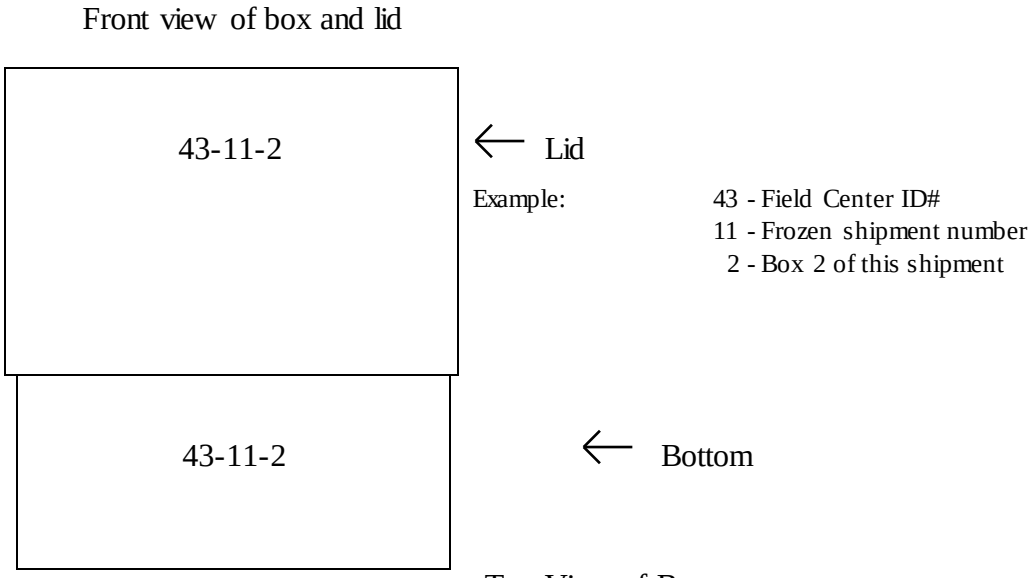
Inventory the processed cryovials in labeled freezer storage boxes as described below.

1. Assign a sequential frozen shipment number to each frozen shipment you send to Fisher BioServices, starting with number "1". Do not use duplicate numbers for two batches since Fisher BioServices cannot track duplicate batch numbers. Do not skip numbers, since the skipped numbers indicate a missing or lost batch.
2. Label any side of both the freezer storage box and lid with your FC number, frozen shipment number, and a box sequence number. The side you label becomes the front of the box. Start the box sequence number with "1" for each month's shipment. For example, use "43-011-2" for the FC with an ID number of 43, frozen shipment number 11, and box number 2.

3. Make a map of aliquot storage in each storage box. For this map, the positions in the storage box are numbered from 1 to 81, starting with the front left, and running from left to right in rows from front to back. See *Figure 7.5 - Specimen Storage Box*. You can map the cryovials in any order in the box. Cryovials from multiple participants can be mapped to one box. Cryovials for the same participant can be mapped to different boxes as long as a shipment to Fisher BioServices includes the samples for both NPAAS Visit 1 and NPAAS Visit 2 for any given participant. The map can help you locate a sample if necessary.
4. Place the frozen cryovials in the freezer storage box according to the map you made.
5. Place the storage box with specimens in the -70°C freezer.

   If after freezing, you find a cryovial is broken, do not send it to Fisher BioServices. Place it in the biohazard container. Record the information of the breakage on a log sheet and edit the corresponding NPAAS Visit Form to indicate that you are not sending the broken sample to Fisher. A broken cryovial may indicate more than 1.8 ml of serum was added to the cryovial. Review the blood processing procedures to ensure blood processing staff do not overfill the vials.

Figure 7.5
Specimen Storage Box



Top View of Box

73								81
64								72
55								63
46								54
37								45
28								36
19								27
10								18
1	2	3	4	5	6	7	8	9

Front

Numbers indicate position numbers inside of storage box

7.5.4.4. Packaging Instructions

Pack the freezer storage boxes of frozen blood and urine samples in the shipping boxes as follows: (The shipping box is composed of an inner styrofoam insulating portion and an outer fiberboard shell). On the day before the shipment, notify Fisher BioServices (via email) of the shipment.

1. Wrap the freezer storage boxes containing the NPAAS samples in absorbent material (paper towels, wadding, etc.).
2. Place the freezer storage boxes in a plastic bag, either self-sealed zip-lock bag or secured with a waterproof seal. Remove as much of the air from the bag as possible and seal.
3. Place dry ice nuggets on the bottom. Place the freezer storage boxes on top of the dry ice layer and then scatter additional dry ice nuggets around the freezer storage boxes. The amount of dry ice used should be consisent with local FC procedures and should be sufficient to ensure that the package will remain frozen for 48 hours, in case the shipment is delayed.
4. Stuff any empty space in the shipping box with newspaper or absorbent material.
5. Place the styrofoam cover on the insulating portion of the shipping box with a copy of the email notification of shipment taped to the top. DO NOT TAPE the styrofoam cover to allow the dry ice gas to escape. Close and tape the outer fiberboard shell of the shipping box by sealing the top and corners of the box with waterproof tape to keep as much cool air as possible from escaping. Do not use scotch or masking tape.
6. Attach the airbill holder to the front of the box. Place the following labels on the top of the shipping box, so that none of the labels touch each other.

Label	Location on Shipping Box
Field Center's return address label	Top, upper left corner
Fisher BioServices address label	Top, lower right corner
Black-and-white class "9" label	Top, under Return address
Priority overnight label	Top, upper right corner
UN3373 Label	Anywhere on top
Biological Substance Category B Label	Anywhere on top
Keep Frozen label (optional)	Anywhere on top

See *Figure 7.6 Frozen Specimen Shipping Labels* to see sample labels and placement of the labels on top of the shipping box. You must follow the current federal regulations of labeling or face a possible fine of up to $10,000 if proper labels are not in compliance.

7. Write in the amount of dry ice placed in the box on the black-and-white class "9" label. Record the weight in pounds or kilograms. Federal Express occasionally changes the weight units on the label. Record the weight in the units requested on the label. You can use the table below to convert from one unit to the other. Federal Express will return shipments which do not show the weight of dry ice.

Pounds	Kilograms	Pounds	Kilograms
10	4.4	16	7.3
11	4.8	17	7.7
12	5.3	18	8.2
13	5.7	19	8.6
14	6.2	20	9.1
15	6.6		

8. Complete the airbill for your carrier. Enter:

   - The date of the shipment.

- Weight of dry ice (in pounds or kilograms), as requested.
- Number of packages and total weight of shipment.

As with the dry ice label, record the weight requested on the label. Insert the airbill in the airbill holder.

9. To delay thawing, place the box in the -70°C freezer to wait for pick up. You don't need to do this if you pack the box within 2-3 hours of pickup and you hold the box in a controlled temperature area (for example, at room temperature and not over 80°F).

10. Notify Fisher BioServices via email (or phone if email is not possible) the day you ship the specimens. Please direct email correspondence to the contacts listed below. Retain a copy of the email notification memo for inclusion in the shipping box. In addition, retain a copy for your records.

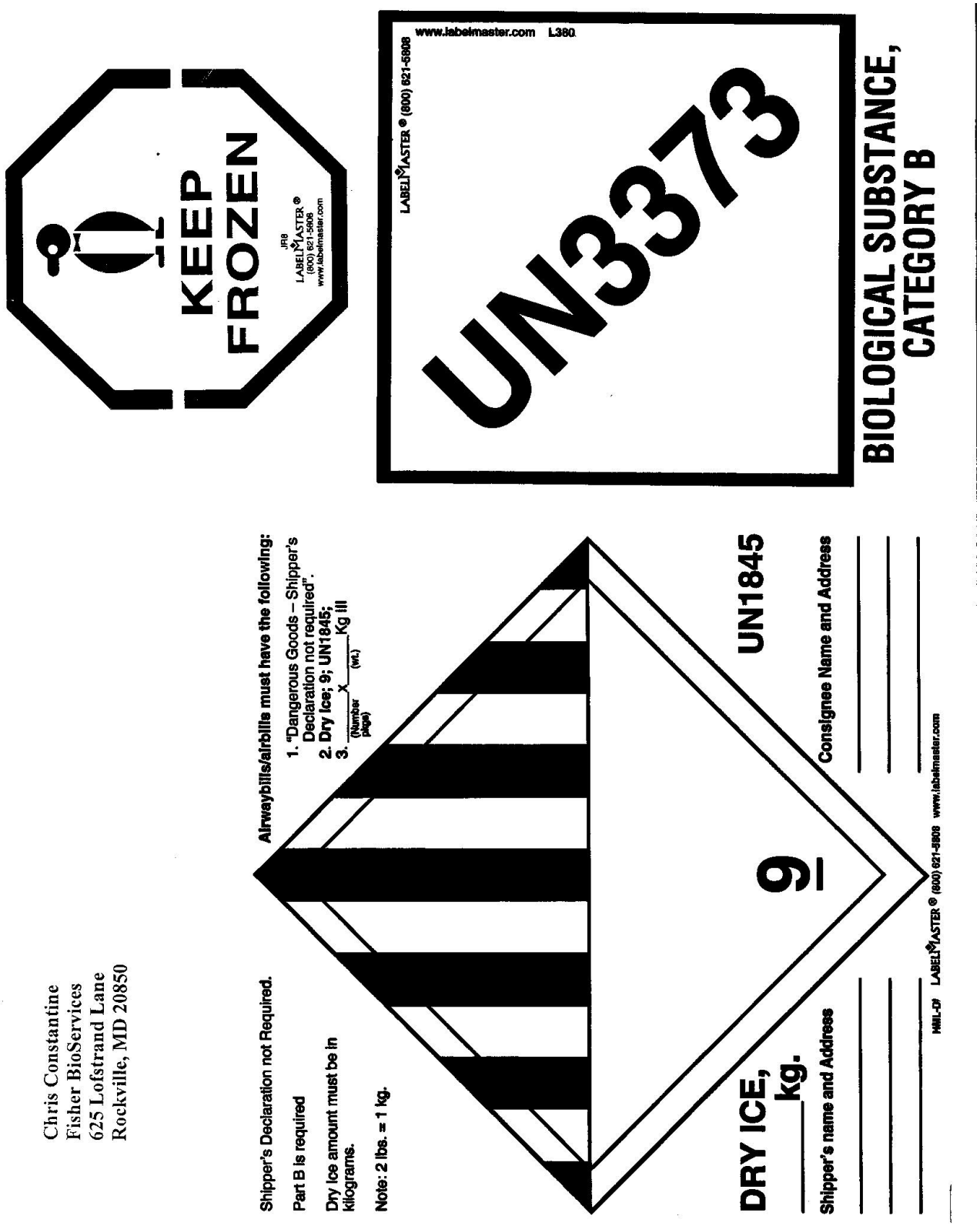
Email Notification of NPAAS Shipment:
Primary Contact *(Include in the "To" line on email correspondence):*
Chris Constantine
Email: chris.constantine@thermofisher.com
Phone: (310) 340-1620

Secondary Contacts *(Include in the "cc' line on all correspondence to Chris Constantine):*
Frank Cammarata: frank.cammarata@thermofisher.com
Brian Sweeney: brian.sweeney@thermofisher.com

In the shipment notification (email) message, include:

- Study Title: WHI NPAAS
- The FC name.
- The date of the shipment.
- The date of expected arrival (same day or next day).
- Number of shippers being sent.
- Number of freezer boxes contained within the shipper(s).
- The airbill number from the carrier's label.

Figure 7.6 Frozen Specimen Shipping Labels



7.5.4.5. Ship frozen NPAAS aliquots to Fisher BioServices every three months.

Ship frozen NPAAS samples to Fisher BioServices. See address below. Packaging and shipping procedures are detailed in the sections that follow.

Chris Constantine
Fisher BioServices,Inc.
WHI Biological Specimen Repository
625 Lofstrand Lane
Rockville, MD 20850
Phone: (310) 340-1620

Ship samples every three months. Ship the first set of samples three months after your first participant has completed NPAAS Visit 1 and NPAAS Visit 2. Ship samples every three months after that regardless of the number of samples in the freezer.
Be sure that all samples from NPAAS Visit 1 and NPAAS Visit 2 are shipped together for a given participant (and similarly for NPAAS Visit 3 and NPAAS Visit 4 for the reliability subsample).

Ship only on a Monday, Tuesday or Wednesday. Do not ship on a day before a Fisher BioServices holiday. Fisher BioServices holidays are : New Year's Day, President's Day, Memorial Day, Independence Day, Labor Day, Columbus Day, Veteran's Day, Thanksgiving, and Christmas.

Samples must be frozen at least two hours at -70°C before packing them for shipment. If the blood samples were drawn on the same day you plan to ship them to FisherBioservices, to help with freezing, leave the lid off the freezer box until it is ready to be packed for shipment. If the insulated shipping container is not filled, stuff newspapers to stabilize the freezer boxes.
Ship the fiberboard shipping box with frozen samples to Fisher BioServices by day or overnight courier to ensure receipt at Fisher BioServices within 24 hours.
There is no minimum number of cryovials to include in a shipment; ship all frozen cryovials regardless of the number of specimens that have been frozen and stored within the last collection period. However, FCs must follow current International Air Transport Association (IATA) regulations. As of January 1997, IATA Dangerous Goods Regulations have changed. The maximum allowable volume of diagnostic specimens allowed in a shipping container has increased from 500 ml to 4 liters (4000 ml). You can send up to 16 freezer boxes in the large shipping container. Include all the blood aliquots you have drawn and frozen.

Notify Fisher BioServices (via email) the day before an NPAAS Shipment. Instructions for sending the email notification are provided in the *NPAAS Manual, Section 7.5.4.4 – Packaging Instructions*. Upon receiving the sample shipment, Fisher will complete send an email to the FC confirming the receipt of the shipment. If the required email notification of NPAAS shipment is not sent to Fisher by the FC, then Fisher cannot send a confirmation of receipt.

Receipt of Frozen Specimen Shipment at Fisher BioServices
Fisher BioServices personnel will email a Confirmation of Receipt to the FC upon arrival of the shipment at the repository.
The Fisher BioServices staff will include:

- Date shipment arrived.
- Number of boxes received.
- Condition of the total shipment, for example, "thawed."

If Fisher BioServices staff do not receive the shipment on the expected day, they will trace the shipment using the airbill number.

SECTION 8
DATA MANAGEMENT

8.0 Introduction

- This section provides information about NPAAS data management: NPAAS label sets, NPAAS data collection forms, NPAAS data entry, and NPAAS WHIX Upgrade Notes.

8.1 NPAAS Label Set

- The CCC provides FCs with NPAAS label sets. These label sets are generic (any specimen ID set can be given to a participant), but once a label set has been used for a participant it is critical that the same specimen ID is used on all the participant's forms and specimens for NPAAS Visit 1 and NPAAS Visit 2.

- Each participant has one NPAAS label set that includes labels for NPAAS Visit 1 and NPAAS Visit 2. For information about the NPAAS label sets, refer to *NPAAS Manual, Section 7.1.6 – Labels*.

8.2 NPAAS Data Collection Forms

- For copies of NPAAS forms, NPAAS forms instructions, and NPAAS worksheets, refer to *NPAAS Manual, Section 9 – NPAAS Forms and Worksheets*.

8.2.1 Notes on NPAAS Forms

- Use the procedures described below to document and handle notes/responses on NPAAS Visit forms, worksheets and/or the participant's *Record Sheet for 24-hour Urine Collection*.

8.2.1.1 Using the "Notes" Box' on the NPAAS Forms

- Record notes/responses on NPAAS Visit forms, worksheets and/or the participant's *Record Sheet for 24-hour Urine Collection* in the following situations:
  - There are missing tasks on the NPAAS Visit forms (*Form 175, Form 176, Form 177, or Form 178)*.
    - If a specific NPAAS task is missed or cannot be completed (i.e., woman refuses a blood draw or is unable to provide a specific spot urine, even after having additional water), NPAAS staff leave the appropriate question on the NPAAS form 'blank' and indicate the problem in the "Notes" box at the end of the NPAAS Visit form.
  - There are out-of-sequence tasks that prevent completion of the NPAAS Visit form (*Form 175, Form 176, Form 177, or Form 178)*.
  - The participant was not given PABA (B-vitamin) tablets because of past hypersensitivity (*Form 175, Form 177)*.
  - There are responses on *NPAAS Visit 2 (or Visit 4) Participant Update Worksheet* indicating potential changes that could affect DLW results (questions 1-3).
  - There are responses on the *Record Sheet for 24-hour Urine Collection* indicating missing or contaminated 24-hour urine collections (questions 1, 2, 4, 5, 6 and 7).

- Refer to *NPAAS Manual, Section 8.2.1.2 – Handling Notes on NPAAS Forms* for information about handling notes recorded on NPAAS forms.

8.2.1.2 Handling Notes on NPAAS Forms

- If staff have a NPAAS Visit form that is incomplete or if staff or participants record notes as described in *Section 8.2.1.1 - Using the "Notes" Box on the NPAAS Forms*), then NPAAS staff should contact the CCC using the steps outlined below:
  1. Make a copy of the entire NPAAS Visit form, *Visit 2 (or Visit 4) Participant Update Worksheet*, or the *Record Sheet for 24-hour Urine Collection* that contains notes or comments.
  2. Black out the participant's name (leave her participant member ID number).
  3. Contact Lynn Fleckenstein at the CCC by email (lflecken@whi.org) or phone (206.667.2946) to let her know that you are sending a FAX.
  4. Fax a copy of the entire NPAAS Visit form, *Visit 2 (or Visit 4) Participant Update Worksheet*, or the *Record Sheet for 24-hour Urine Collection* to Lynn Fleckensteinat the CCC (Fax #: 206.667.4142). Please use a cover sheet, as the fax number serves the entire CCC.

8.2.2 Management of NPAAS Forms

- During NPAAS Visits, it is critical that all the pages of NPAAS forms be kept together. The participant member ID is only on the first page of the NPAAS form, thus it is critical that NPAAS staff not separate the pages. The form needs travel with the participant.
  - For example, the 3-hour urine/blood specimens; staff should walk the participant to the urine collection area and then wait to escort her (and her NPAAS form) for the 3-hour blood draw.

- All completed NPAAS forms (i.e., *Form 174 – NPAAS Screening Result, Form 175 – NPAAS Visit 1*, etc.) and NPAAS worksheets (i.e., *NPAAS Visit 1 Eligibility Worksheet, NPAAS Visit 2 Participant Update Worksheet*, etc.) are placed in the participant's chart (or special NPAAS notebook), even when a participant is ineligible or has declined participation.
  - For example: *Form 174 – NPAAS Screening Result* should be filed in the participant's chart (or special NPAAS notebook) regardless of the screening outcome (i.e., Scheduled, Ineligible or Declined).

8.3 Data Entry

- The NPAAS data forms are entered at the CCC.

8.3.1 Trouble Shooting Forms Management

- This section provides information to help trouble shoot management of NPAAS Visit forms.

8.3.1.1 NPAAS Labels Do Not Match

- If a participant's NPAAS label on *Form 175 – NPAAS Visit 1* and *Form 176 – NPAAS Visit 2* are NOT the same identification number, the FC needs to:
  - Resolve the problem (if it is an obvious problem) by re-labeling all the woman's specimens with a new label set for the two visits (NPAAS Visit 1 and NPAAS Visit 2).
  - Contact the CCC to help work through the problem.

8.3.1.2 Field Omissions

- WHIX will not allow a NPAAS Visit form (*Form 174, 175, 176, 177, 178*) to be data entered if certain information is omitted or missing on the form(s). To help prevent potential field omissions and quickly resolve omissions that do occur, FCs are asked to use the thoroughly review the forms and ensure that all required NPAAS tasks are completed.

  Completeness Checklist and Completeness Staff IDs*:*

- *Form 175 - NPAAS Visit* 1, *Form 176- NPAAS Visit 2*, *Form 177 - NPAAS Visit 3*, and *Form 178- NPAAS Visit 4* have a 'Completeness Checklist' that helps staff review the form. It is important for the NPAAS-Lead ops (or designee) to review the form and check to see that all NPAAS visit activities have been completed and the information has been entered correctly on the form. The Lead-ops (or designee) reviewing *Forms 175, 176, 177,* and *178* enters their Staff ID on the last page the form, *Completeness Staff ID* to indicate that the form has been reviewed and checked for completeness.

8.3.1.3 Time Sequence of NPAAS Visit Activities

- Spot urines, DLW dosing, meal replacement beverage, and intake of other beverages must be completed in the sequence specified on the NPAAS Visit form.

SECTION 9
NPAAS FORMS AND WORKSHEETS

9.0 Overview

- This section provides copies of the NPAAS study forms and worksheets. Accompanying each form is a detailed set of instructions describing who completes the form, when and how each data item should be coded, and what should happen to the form when it is completed. Copies of the following NPAAS forms and worksheets are included in this section.

9.1 Printing NPAAS Forms and Worksheets

- FCs will receive 'PDF' files of all NPAAS Visit forms (i.e., *Forms 174, 175, 176, 177, 178, and 179*) and NPAAS Worksheets (i.e., *NPAAS Visit 1 Eligibility Worksheet, NPAAS Visit 2 Participant Update Worksheet, NPAAS Visit 3 Eligibility Worksheet,* and *NPAAS Visit 4 Participant Update Worksheet*). FCs use these electronic files or the hard copies available in the *NPAAS Manual, Section 9 – NPAAS Forms and Worksheets* to make copies of forms and worksheets, as needed. For information about NPAAS printed materials, refer to *NPAAS Manual, Section 2.4.1 – Print Materials*.

Forms & Worksheets – NPAAS Visit 1 and NPAAS Visit 2:

- *NPAAS Visit 1 Eligibility Worksheet*
- *Form 35 – Personal Habits Update*
- *Form 35* Forms Instructions
- *Form 45 – Current Supplements (Back-Up)*
- *Form 45* Forms Instructions
- *Form 60 – Food Frequency Questionnaire (FFQ)*
- *Form 60* Forms Instructions
- *Form 61 – How to Fill Out the Food Questionnaire*
- *Form I71 – Viewpoints*
- *Form 171* Forms Instructions
- *Form 172 – Seven Day Physical Activity Recall (PAR)*
- *Form 172* Forms Instructions
- *Script for Physical Activity Recall (PAR)*
- *Arizona Activity Frequency Questionnaire (AAFQ)*
- *AAFQ* Forms Instructions
- *Four Day Food Record (Multiple Day Food Record) (4DFR)*
- *Four Day Food Record* Forms Instructions
- *Four Day Food Record Worksheet for Staff*
- *Portion Size Booklet (Serving Size Booklet)*
- *24-Hour Urine Collection Worksheet for Staff*
- *Form 174 – NPAAS Status Update*
- *Form 174* Forms Instructions
- *Form 175 – NPAAS Visit 1*
- *Form 175* Forms Instructions
- *NPAAS Visit 2 Participant Update Worksheet*
- *Form 176 – NPAAS Visit 2*
- *Form 176* Forms *Instructions*

Forms & Worksheets – NPAAS Visit 3 and NPAAS Visit 4 (Reliability Subsample):

- *Form 35 – Personal Habits Update*
- *Form 35* Forms Instructions

- *Form 45 – Current Supplements (Back-Up)*
- *Form 45* Forms Instructions
- *Form 60 – Food Frequency Questionnaire (FFQ)*
- *Form 60* Forms Instructions
- *Form 61 – How to Fill Out the Food Questionnaire*
- *Form I71 – Viewpoints*
- *Form 171* Forms Instructions
- *Form 172 – Seven Day Physical Activity Recall (PAR)*
- *Form 172* Forms Instructions
- *Script for Physical Activity Recall (PAR)*
- *Arizona Activity Frequency Questionnaire (AAFQ)*
- *AAFQ* Forms Instructions
- *Four Day Food Record (Multiple Day Food Record) (4DFR)*
- *Four Day Food Record* Forms Instructions
- *Four Day Food Record Worksheet for Staff*
- *Portion Size Booklet (Serving Size Booklet)*
- *24-Hour Urine Collection Worksheet for Staff*
- *Pre-Visit 3 Eligibility Worksheet*
- *NPAAS Visit 3 Eligibility Worksheet*
- *Form 177 –NPAAS Visit 3*
- *Form177* Forms Instructions
- *NPAAS Visit 4 Participant Update Worksheet*
- *Form 178 – NPAAS Visit 4*
- *Form 178* Forms Instructions

WHI-ES NUTRITION AND PHYSICAL ACTIVITY ASSESSMENT STUDY (NPAAS)
PROCEDURE MANUAL

TABLE OF CONTENTS

SECTION 3: RECRUITMENT

SECTION 4: SCREENING

SECTION 5: NPAAS VISITS

Figures

SECTION 6: NPAAS RELIABILITY SUBSAMPLE

Figures

SECTION 7: BLOOD AND URINE COLLECTION, PROCESSING, AND SHIPMENT

Figures

SECTION 8: DATA MANAGEMENT

SECTION 9: NPAAS FORMS AND WORKSHEETS

Forms & Worksheets – NPAAS Visit 1 and NPAAS Visit 2:

- *NPAAS Visit 1 Eligibility Worksheet*
- *Form 35 – Personal Habits Update*
- *Form 35* Forms Instructions
- *Form 45 – Current Supplements (Back-Up)*
- *Form 45* Forms Instructions
- *Form 60 – Food Frequency Questionnaire (FFQ)*
- *Form 60* Forms Instructions
- *Form 61 – How to Fill Out the Food Questionnaire*
- *Form I71 – Viewpoints*
- *Form 171* Forms Instructions
- *Form 172 – Seven Day Physical Activity Recall (PAR)*
- *Form 172* Forms Instructions
- *Script for Physical Activity Recall (PAR)*
- *Arizona Activity Frequency Questionnaire (AAFQ)*
- *AAFQ* Forms Instructions
- *Four Day Food Record (Multiple Day Food Record) (4DFR)*
- *Four Day Food Record* Forms Instructions
- *Four Day Food Record Worksheet for Staff*
- *Portion Size Booklet (Serving Size Booklet)*

- *24-Hour Urine Collection Worksheet for Staff*
- *Form 174 – NPAAS Status Update*
- *Form 174* Forms Instructions
- *Form 175 – NPAAS Visit 1*
- *Form 175* Forms Instructions
- *NPAAS Visit 2 Participant Update Worksheet*
- *Form 176 – NPAAS Visit 2*
- *Form 176* Forms *Instructions*

Forms & Worksheets – NPAAS Visit 3 and NPAAS Visit 4 (Reliability Subsample):

- *Form 35 – Personal Habits Update*
- *Form 35* Forms Instructions
- *Form 45 – Current Supplements (Back-Up)*
- *Form 45* Forms Instructions
- *Form 60 – Food Frequency Questionnaire (FFQ)*
- *Form 60* Forms Instructions
- *Form 61 – How to Fill Out the Food Questionnaire*
- *Form I71 – Viewpoints*
- *Form 171* Forms Instructions
- *Form 172 – Seven Day Physical Activity Recall (PAR)*
- *Form 172* Forms Instructions
- *Script for Physical Activity Recall (PAR)*
- *Arizona Activity Frequency Questionnaire (AAFQ)*
- *AAFQ* Forms Instructions
- *Four Day Food Record (Multiple Day Food Record) (4DFR)*
- *Four Day Food Record* Forms Instructions
- *Four Day Food Record Worksheet for Staff*
- *Portion Size Booklet (Serving Size Booklet)*
- *24-Hour Urine Collection Worksheet for Staff*
- *Pre-Visit 3 Eligibility Worksheet*
- *NPAAS Visit 3 Eligibility Worksheet*
- *Form 177 –NPAAS Visit 3*
- *Form177* Forms Instructions
- *NPAAS Visit 4 Participant Update Worksheet*
- *Form 178 – NPAAS Visit 4*
- *Form 178* Forms Instructions

APPENDICES