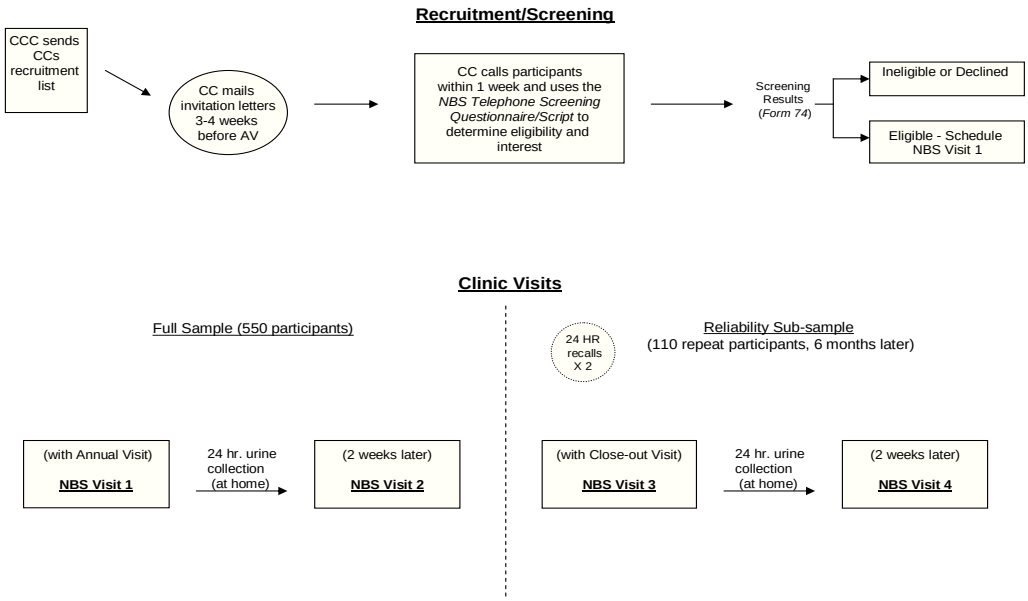
SECTION 1
WHI NUTRITIONAL BIOMARKERS STUDY OVERVIEW

1.0 Overview of the WHI Nutritional Biomarkers Study (NBS)

- The WHI Nutritional Biomarkers Study (NBS) is a substudy of the WHI. The objective of the NBS is to collect biological markers of dietary intake (energy, protein, fatty acids, and micronutrients) for use in regression calibration models that will correct the random and systematic bias of dietary self-report and better estimate associations of dietary intake with disease outcomes. The calibrated nutrient intake data will be used for a broad range of nutrient-disease analyses from the WHI. For a copy of the NBS protocol, refer to *NBS Manual*, *Appendix A – NBS Protocol (ver.2)*.

- NBS will be implemented in a subset of 550 DM women (275 Intervention, 275 Control). DM women will be mailed an invitation letter prior to their regularly scheduled Annual Visit (AV). Willing participants will be consented at the first clinic visit (5 hours), complete an FFQ, provide information about current dietary supplements (*Form 45*) and physical activity (*Form 35*), 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 return to their CC for a 2-hour visit on about Day 15, at which time they will return a 24-hour urine specimen (collected at home), be weighed, provide a fasting blood specimen, and two spot urines. For a subset of 110 women (the reliability subsample), the full protocol will be repeated in conjunction with a woman's close-out visit, an average of 6 months later. Women in this reliability subsample will also complete two 24-hour recalls (24-HR), one within three weeks of completing the study the first time and the second within about three weeks of the study the second time. The Nutritional Biomarkers Study implementation begins May 2004 and continues through March 2005. Figure 1.1 – *WHI Nutritional Biomarkers Study Overview* provides an overview of NBS.

Figure 1.1
WHI Nutritional Biomarkers Study Overview



SECTION 2
GUIDELINES

2.0 Overview

- This section contains information to help Clinical Centers manage the Nutritional Biomarkers Study. Guidelines for staffing, training and certification, 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 CC has the following staff positions covered in their initial NBS budget projections: Nutritionist, Medical Assistant, Research Assistant, and Lab Technician.

Blinding

- Ideally staff responsible for data collection (i.e., height/weight, forms review, blood and urine processing) should be blinded to the DM randomization assignment of NBS participants.

2.1.1 NBS Lead-Ops

- Each CC identifies a NBS Lead-ops person to oversee NBS operations and be the NBS point person. This person may be the CC Principal Investigator, the investigator with local oversight for the NBS, or a separate person. The NBS Lead-ops person needs to:
  - Be in the clinic and involved in the NBS visits so that they can supervise what is happening and respond to questions that staff may ask.
  - Train other non-lead NBS staff.
  - Ensure that the NBS protocol is followed, data entry is timely, supplies are available and any procedural or data entry problems are reported to the CCC.
  - Oversee management of the following NBS operations:
    - Recruitment
    - Integration of NBS visit tasks with AV and WHI Close-out tasks
    - NBS visits and tasks
    - CC-NBS budget

2.2 Training and Certification

- The purpose of training within NBS is to teach staff the NBS protocol and procedures for implementation at the Clinical Center (CC). To facilitate the implementation of NBS, the CCC uses a training model similar to the one used in WHI. One NBS Lead-ops person per CC receives training for all NBS-specific tasks and is then responsible for training other staff for NBS tasks. The CCC provides training conference calls, the *NBS Manual*, and NBS Staff Training and WHI Certification Guidelines (refer to *NBS Manual*, *Appendix E – NBS Training Materials*).

2.2.1 NBS Lead-Ops Person

- The NBS Lead-ops person attends each of the four NBS training calls and reads the *NBS Manual*.
  - If the Lead NBS-ops person cannot attend a NBS training call, 'essence' transcriptions of the calls are available. To be trained and certified, the Lead NBS-ops person reviews the transcript of the training call(s) and reads the *NBS Manual*.

2.2.2 Non-Lead NBS Staff

- Non-lead NBS staff read relevant sections of the *NBS Manual* and discuss relevant training call topics with the NBS Lead-ops person. To help guide training for non-lead NBS staff, copies of the 'essence' transcriptions of the four NBS training calls are available in the *NBS Manual*, *Appendix E – NBS Training Materials.*

- For WHI tasks that are part of the NBS (i.e., physical measures, FFQ review, blood drawing and processing, forms review, and data entry), the CCC asks that CCs use WHI-certified staff. For guidelines on which tasks need WHI certification, refer to *WHI Certification Guidelines* in the *NBS Manual*, *Appendix E – NBS Training Materials*.

- For training on NBS-specific urine collection and processing procedures, the NBS Lead-ops person attends the NBS training calls and reads the *NBS Manual*. The Lead-ops person trains the designated CC staff. CCs must also follow the pertinent safety precautions found in the *NBS Manual*, *Section 7.1.2– Safety Procedures – Precautions for Handling Urine*, as well as their local regulations for bio-specimen handling (blood and urine).

2.2.3 Training Resources

2.2.3.1 NBS Training Conference Calls

- The CCC conducts four NBS training conference calls at the time of NBS start-up.
  - Call #1: provides an introduction and overview of NBS (purpose of study, recruitment, visit tasks and housekeeping issues for start-up).
  - Call #2: focuses on the doubly labeled water (DLW) protocol.
  - Call #3: focuses on specimen collection and processing of spot and 24-hour urine and blood biomarkers, as well as forms and data entry.
  - Call #4: provides an opportunity to review and discuss the overall NBS process.

- The *NBS Manual*, *Appendix E – NBS Training Materials* includes the agenda and 'essence' transcription for each training conference call.

2.2.3.2 NBS Procedure Manual (referred to as the *NBS Manual*)

- The *NBS 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 NBS clinics as a way of assuring the quality of the study.

2.3 Communication

2.3.1 Email Communication

- The following types of email communication are sent to the CC PI, all NBS CC investigators, and individuals identified to receive NBS mailings (i.e., NBS Lead-ops person).
  - **NBS Updates:** A weekly email to maintain systemic communication and keep NBS clinics informed.
  - **NBS Alerts**: Priority email sent when there is immediate information that is important to quickly relay to all NBS clinics.

- CCs send NBS-related questions to Helen Penor (hpenor@whi.org) for triaging to the appropriate CCC staff for a response.

2.3.2 WHI Public Folders

- The Outlook Public Folders contain a *Nutri Biomarker Study* folder for NBS-specific materials and communications. This folder provides resources for the implementation of the NBS. The *Nutri Biomarker Study* folder includes items such as:
  - NBS WHILMA Upgrade notes.
  - NBS Updates – copies of all NBS weekly updates published to date.
  - NBS Alerts – copies of all NBS Alerts published to date.
  - NBS materials submitted to the FHCRC IRB – copies of materials submitted and approved by the FHCRC IRB.
  - NBS Timeline.
  - NBS Study Materials – electronic copies of all data collection forms, worksheets and participant materials unique to NBS.
  - NBS Q&A about Study Procedures

2.4 Supplies

- This section provides information about supplies for the WHI Nutritional Biomarkers Study (NBS).

2.4.1 Supplies Obtained by Each CC

- Each CC obtains the supplies shown below. The CCs will receive reasonable reimbursement for items necessary to complete the study.

Urine Collection: [1]

- Gel ice packs
- Safety pins

Miscellaneous: [1]

- Participant meal replacement beverage
- Participant lunch
- Participant snack
- Straws
- Ziplock baggies

Print Materials

CCs print NBS forms and materials locally from masters provided by CCC. Electronic copies of the masters are posted in the Outlook Public Folders/Nutri Biomarkers Study/NBS Study Materials. Hard copies of the masters are also available in the *NBS Manual (Section 9 – NBS Forms and Worksheets, Appendix C – NBS Participant Materials* and *Appendix D – NBS Staff Materials).* The NBS forms and materials that CCs print locally are outlined below.

- *Letter of Invitation to Participants*
- *Telephone Screening Questionnaire/Script*
- *Frequently Asked Questions* (for staff)
- *Study at a Glance*
- *NBS Consent*
- *Instructions for 24-hour Urine Collection*
- *NBS Visit Forms* (*Forms 74 - 78*)
- *NBS Visit 1 Eligibility Checklist Worksheet*
- *NBS Visit 2 Participant Update Worksheet*
- *NBS Visit 2 (or Visit 4) PABA Worksheet for Staff*
- *NBS Visit 3 Eligibility Checklist Worksheet*
- *NBS Visit 4 Participant Update Worksheet*
- *Consent for Future Contac*t and *Continued Participation in this Study*

Other Supplies (WHI supplies used for NBS):

- Specimen collection and processing supplies:[1]
  For example: Royal blue-stoppered serum tubes, Lavender-stoppered plasma tubes, 2.0 mL cryovials, gloves, needles, disposable pipette tips.
- Office supplies (e.g., envelopes, mailing labels, member ID labels, postage)

[1] For information about recommended vendor/part number and quantities to purchase, refer to *NBS Manual, Section 2.4.4 – Guidelines for Ordering Supplies*.

2.4.2 Supplies Provided by the CCC

- The CCC provides each CC with the supplies outlined below.

Forms & Folders & Labels

- WHI Folders
- NBS label set
- WHI Form 35
- WHI Form 45 (Back-up)
- WHI Form 60 [2]

Urine Collection:

- Urine collection hats
- 24-hour urine collection containers (3 liter)
- Plastic funnels
- Sturdy plastic bags with a handle

For participants who receive PABA:

- PABA (B-vitamin) tablets, 3 per participant
- Boric acid powder (preservative for the 24-hour urine collection)
- Stickers for 24-hour urine collection containers

Urine Processing:

- 5 mL Corning cryovial tubes with rubber O-ring seal
- 250 mL graduated cylinders
- 10 mL tubes (for centrifuging 24-hour urine collection sample)
- Disposable pipettes (for urine)

Urine Storage and Shipping:

- Freezer boxes with dividers for the 5 mL Corning cryovial tubes

[2] Note: The CCC will provide Form 60 (Ver. 1.5) which can only be marked for Visit Type AV1 – AV9. CCs will use Form 60 (Ver. 1.6) from their local supply if an NBS participant is at AV10 or AV11 and part of the FFQ subsample. FFQ Ver. 1.5 and Ver. 1.6 are identical except that Ver. 1.6 can be marked for Visit Type AV10 or AV11.

2.4.3 Supplies Provided by Dale Schoeller (University of Wisconsin)

- Dale Schoeller at the University of Wisconsin provides each CC with the supplies outlined below.

Doubly Labeled Water (DLW) Protocol:

- DLW (in pre-measured bottles)
- Pre-weighed tissues (for use in case of DLW spillage)

2.4.4 Guidelines for Ordering Supplies

- The list below includes guidelines for ordering miscellaneous supplies and supplies for blood and urine collection, processing, and shipment. CCs may choose to use a different vendor than the one recommended for supplies obtained locally. Please note that blood collection supplies may be ordered through McKesson BioServices, as indicated.

Miscellaneous Supplies	**Recommended Vendor/Part Number:**
NBS-specific:	
• Participant meal replacement beverage (8 ounce) for 1 hour after DLW dose at Visit 1 1 per NBS participant	Local medical supply or store Suggested brand: Boost (based on informal taste testing at the CCC)
• Participant lunch at the end of Visit 1 1 per NBS participant	CC option
• Participant snack at the end of Visit 2 1 per NBS participant	CC option
• Straws (for drinking DLW) 2 per NBS participant	Local medical supply or store (a small size that can be pushed all the way inside the DLW dose bottle)
• Ziplock baggies (for use with pre-weighed tissues in case of DLW spillage) 1 box (of ~50) per CC	CC to purchase at local store
Blood and Urine Collection Supplies	
NBS-specific:	
• Urine collection hats (Specipans) 3 per NBS 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
• 24-hour urine collection containers 2 (3 liter) per NBS participant	CCC will order and provide
• Plastic funnels for 24-hour urine collection 1 per NBS participant	CCC will order and provide
• Plastic carry bag for 24-hour urine collection kit 1 per NBS participant	CCC will order and provide
• Safety pins (large size) for participants to pin to their undergarments as a reminder for 24-hour urine collection 1 per NBS participant (1 box of ~100 per CC)	Local store
• Gel ice packs for participants to transport their 24-hour urine collections to the Clinical Center 20 per CC (~6 ounce; 4" x 6")	Local medical supply or store
For participants who receive PABA	
• PABA (B-vitamin) tablets, 3 per participant	CCC will order and provide
• Boric acid powder, one 500 gram jar	CCC will order and provide
• Stickers for 24-hour urine collection containers	CCC will order and provide

Blood and Urine Collection Supplies	**Recommended Vendor/Part Number:**
Same as WHI (use from WHI stock and order as usual to replenish supplies):	
• Royal blue-stoppered serum tubes for trace elements and carotenoids, no additive, silicone coated, 7 mL (***Note:*** **7 mL will be printed on label in red ink; be sure you use the tubes with 7 mL in red and not 7 mL in green)** 3 per NBS participant	McKesson or BD 367737 (Baxter B2951-107, Vacutainer® tubes with hemoguard closure) or BD 6526 (Baxter B3007-54)
• Lavender-stoppered plasma 10 mL tube, with **powdered Sodium (Na_2 EDTA)** 1 per NBS participant under the age of 60 years 2 per NBS participant 60 years of age and older	McKesson or Monoject 310745 Terumo Venoject T200SQ (Baxter B3042-54 or Laboratory Supply)
• Needles, 21 gauge, 1 to 1½" multiple sample Vacutainer®	Local medical supply
• 23-gauge butterfly with 12" tubing with multiple sample Luer adapter	Local medical supply
• Syringes	
• Monoject hypodermic needle (21-23 gauge, 1 ½")	
• Blood draw workstation	Recommended Baxter S9267-1
• Vacutainer® holder	Local medical supply
• Test tube racks	Local medical supply
• Alcohol swabs or cotton balls, alcohol, and alcohol dispenser or gauze swabs	Local medical supply
• Bandages ("Band-Aids") or surgical tape	Local medical supply
• Biohazard container for needles ("sharps" container)	Local medical supply
• Biohazard container for waste	Local medical supply
• Wash bottle	Local medical supply
• Lab coat	Local medical supply
• Disposable latex gloves	Local medical supply
• Chlorine bleach	Local store
• Anti-bacterial hand soap	Local store
• Aluminum foil or yellow plastic sleeves to protect the blood drawn in **all** of the blood collection tubes from light (NBS Visit 2)	Local store

Blood and Urine Processing Supplies	**Recommended Vendor/Part Number:**
NBS-specific:	
• 5 mL Corning cryovial tubes with rubber O-ring for processing spot urine samples 6 per NBS participant 12 additional for each participant who has two additional aliquots taken at each time point for QC (for QC blind duplicates, aliquot one extra vial for each collection for each of 30 participants NBS-wide)	CCC will order and provide
• 250 mL graduated cylinder to measure 24-hour urine samples 2 per CC	CCC will order and provide
• 10 mL tube for centrifuging 24-hour urine collection samples 1 per participant per CC plus 10 extra	CCC will order and provide
• Disposable transfer pipettes (for transferring urine from DLW spot urine collection to 5 mL cryovial, and for transferring the 10 mL centrifuged 24-hour urine sample into the three 2 mL cryovials) 7 per participant	CCC will order and provide
Same as WHI (use from WHI stock and order as usual to replenish supplies):	
• 2 mL cryovials for serum, plasma and 24-hour urine 13 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 3 for 24-hour urine	
• Fluid-resistant lab coat	Local medical supply
• Refrigerated centrifuge with swinging buckets (able to reach relative centrifugal force of 1,300 xg)	Local medical supply
• Factory certified low temperature thermometer, -90°C to +20°C or thermistor	
• Large and small test tube racks for holding Vacutainer® tubes and cryovials	Local medical supply
• Disposable latex gloves	Local medical supply
• Chlorine bleach	Local medical supply
• Goggles or glasses or mask with face shield or barrier shield behind which to process blood samples	Local medical supply
• Tape	Local store
• 1 mL adjustable automatic pipettor	Local medical supply

Blood and Urine Processing Supplies (continued)	**Recommended Vendor/Part Number:**
• Disposable pipette tips (for blood processing)	Local medical supply
• 10 x 75 mm or larger test tube or 15 mL conical centrifuge tube to recentrifuge samples	Baxter C3902-4

Blood and Urine Storage and Shipment

NBS-specific:

• Freezer boxes for 5 mL Corning cryovial tubes	CCC will order and provide

Same as WHI (use from WHI stock and order as usual to replenish supplies. Exceptions: freezer, low temperature thermometer, and scale which are pre-existing WHI equipment)

• Freezer at 70°C or colder, with CO2 back-up system and temperature recorder	Pre-existing WHI equipment
• Factory certified low temperature thermometer, -90°C to +20°C or thermistor	Pre-existing WHI equipment
• Newspaper (for packing)	
• Freezer alarm for each -70°C freezer	Rees Scientific/Informer 2400 Telephone: (800) 327-3141
• Waterproof packing tape (strapping tape)	
• Dry ice nuggets	
• Freezer gloves/vinyl or latex rubber gloves	
• Return address label (printed at CC)	
• Indelible ink pen	
• Shipping tape	
• Scale for weighing shipment	Pre-existing WHI equipment

SECTION 3
RECRUITMENT

3.0 Overview

- This section contains information to help clinical centers manage NBS recruitment. The section describes the NBS study sample, recruitment goals, recruitment plan, recruitment list, invitation letter, WHILMA resources for recruitment, and procedures for expanded NBS recruitment (beginning July 2004).

3.1 NBS Study Sample

- NBS will be implemented in a subset of 550 DM women (275 Intervention, 275 Control) with age and race/ethnicity strata that represent the composition of the DM cohort.

3.2 Recruitment Goals

- The recruitment goal for each CC is approximately 50 participants, except the two minority CC sites, which are expected to recruit at least 25 minority participants each.

- Each CC will strive to recruit 25 DM-I participants and 25 DM-C participants (minority sites about 13 DM-I and 13 DM-C participants). CCs do not have goals for the various age and ethnicity strata. The CCC will close strata when the goal for a specific stratum has been reached NBS-wide (e.g., white women age 55-59). Minority cells will remain open for recruitment after the goal has been met to allow as many minorities as possible to participate. Non-minority cells will close after the goal has been met.

3.3 Recruitment Plan

- Two NBS recruitment plans are available as of July 2004:
  - NBS Visit linked with AV
  - Expanded NBS recruitment

<u>NBS Visit Linked with AV:</u>

- The plan for NBS recruitment is to invite and schedule participants for NBS in conjunction with their WHI Annual Visit (AV). Specifically, the plan includes approaching only those participants who are due for their AV during the NBS window, but have not yet completed the AV. This recruitment plan is designed to:
  - Minimize participant burden.
  - Avoid subjectively selecting participants (to avoid selection bias).
  - Ensure that the age and race/ethnic composition of the NBS sample is reasonably representative of the DM cohort.
  - Ensure that women known to have exclusion criteria (e.g., diabetes) do not receive an invitation letter.

- It is important for all CCs to begin recruitment by using the NBS recruitment plan (i.e., approach only participants who are due for their AV during the NBS window, but have not yet completed their AV). The CCC will monitor recruitment rates and will re-evaluate CC recruitment in early July 2004 to see if there is a need to expand the recruitment pool.

<u>NBS Expanded Recruitment:</u>

- For information about expanded NBS recruitment beginning July 2004, refer to the *NBS Manual, Section 3.7 – Expanded NBS Recruitment (Beginning July 2004)*.

3.4 Recruitment List

- The CCC provides each CC with a list of eligible participants by way of the *NBS Recruitment Tracking* report (NBS001). The report includes DM participants (Intervention and Control) likely to have their AV during the NBS window (May-September 2004) and who meet the eligibility criteria outlined in the NBS protocol. The list includes women who meet the following criteria:
  - Have an AV target date between February-September 2004.
  - Have not completed an AV for 2004.
  - Have a FFQ at baseline, Year 1, and at least one additional time point.
  - Have full-follow-up status.
  - Do not take insulin or oral hypoglycemic agents to manage diabetes.
  - Are not part of the WHI blood draw cohort.

- CCs use the *NBS Recruitment Tracking* report (NBS001) to invite and schedule women for NBS in conjunction with their AV. For further details about the recruitment list and the *NBS Recruitment Tracking* report, refer to *NBS Manual, Section 3.6 - WHILMA Resources for NBS Recruitment Tracking* and the *Nutritional Biomarkers Study-Recruitment Upgrade Notes* located in the Outlook Public Folders/All Public Folders/Nutri Biomarkers Study (dated 5/12/04).

- For information about the supplemental list for expanded NBS recruitment, refer to the *NBS Manual, Section 3.7.3 – Supplemental Recruitment List for Expanded NBS Recruitment.*

3.5 Invitation Letter

- After receiving the participant recruitment list from the CCC, CCs begin to mail the NBS invitation letter to participants in weekly batches (i.e., ideally about 3 weeks before the AV, but no less than 2 weeks). A copy of the *NBS Letter of Invitation to Participants* is available the *NBS Manual, Appendix C – NBS Participant* M*aterials*.

- It is important to mail the NBS invitation letter separate from other AV mailings. This provides an opportunity to:
  - Ensure that the invitation letter is not lost in the WHI AV materials.
  - Send the invitation letter in a timely manner (i.e., about 3 weeks before the AV).

- It is important to mail the invitation letter in small batches (i.e., do not to mail to the entire NBS recruitment list all at once). If CCs mail too far in advance, the participant's strata status may change before a CC can contact the participant. Use the 'AV Target Date Between' parameter on the *NBS Recruitment Tracking* report (NBS001) to identify eligible participants due for an upcoming AV. NBS staff can choose an AV target date range to identify participants between XX and XX dates. This will allow CCs to mail smaller weekly batches of invitation letters to eligible participants.

- To avoid any potential of selection bias (subjectively selecting participants), CCs should mail an invitation letter to only the participants appearing on their *NBS Recruitment Tracking* report (NBS001) (i.e., don't add names) and to invite all participants appearing on their *NBS Recruitment Tracking* report (NBS001)unless:
  - They have completed their AV visit.
  - Their strata closes before the CC approaches them.
  - They are known apriori to be ineligible (e.g., live greater than 200 miles distant from the CC). Refer to *NBS Manual, Section 4.1.4 – Complete and Data Enter NBS Screening Result (Form 74).*

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

    For information about a sample Call Attempts Log, refer to *Nutritional Biomarkers Study-Recruitment Upgrade Notes* located in the Outlook Public Folders/All Public Folders/Nutri Biomarkers Study (dated 5/12/04).

- For information about approaching participants for expanded NBS recruitment, refer to the *NBS Manual, Section 3.7.4 – Approaching Participants for Expanded Recruitment.*

3.6 WHILMA Resources for NBS Recruitment Tracking

- This section describes the resources for NBS recruitment tracking. This includes the *NBS Recruitment Tracking* report (NBS001) and the NBS Custom Data Extract (CDE for NBS001). Supporting information can be found in the *Nutritional Biomarkers Study-Recruitment Upgrade Notes* located in the Outlook Public Folders/All Public Folders/Nutri Biomarkers Study (dated 5/12/04).

- For information about the *NBS Supplemental Recruitment Tracking* report (NBS002), refer to *NBS Manual, Section 3.7.3 – Supplemental Recruitment List for Expanded Recruitment.*

3.6.1 NBS Recruitment Tracking Report

- The *NBS Recruitment Tracking* report (NBS001) provides NBS staff with a complete system for tracking NBS recruitment. For information about the report and the parameters that allow the data to be sorted and filtered, refer to *Nutritional Biomarkers Study-Recruitment Upgrade Notes* located in the Outlook Public Folders/All Public Folders/Nutri Biomarkers Study (dated 5/12/04).

  Helpful Hints:
  - NBS staff can run the *NBS Recruitment Tracking* report (NBS001) in two different formats: a 'List' format which provides a list of several participants per page or a 'Call Log' format which lists one participant per page and includes a blank Call Attempts Log. NBS staff may find the Call Attempts Log helpful for tracking their screening call attempts to participants.
  - To ensure that women are invited and scheduled in conjunction with their AV, use the 'Maybe Had AV' column on the *NBS Recruitment Tracking* report (NBS001). A 'yes' in this column, identifies participants who have completed *Form 80* or *Form 33* for the targeted AV. If there is a 'yes', check to see if the woman has already completed her AV. If she has completed her AV, do not mail an invitation letter.
  - CCs should monitor the 'Strata Status' column on the *NBS Recruitment Tracking* report (NBS001) as they prepare their mailings to avoid mailing to participants who should not be contacted because their strata has closed.
  - The *NBS Recruitment Tracking* report (NBS001) can be run with a participant's randomization assignment (i.e., Intervention or Control) either showing or hidden. The ability to 'hide' DM randomization assignment provides flexibility and the option to maintain DM blinding for NBS staff not involved in NBS recruitment.

3.6.2 NBS Custom Data Extract

- The NBS Custom Data Extract (for NBS001) contains the same columns as the NBS Recruitment Tracking report (NBS001), plus some additional information (e.g., a column that flags participants who agree to repeat the NBS protocol in 6 months).

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

3.7 Expanded NBS Recruitment (Beginning July 2004)

- This section describes procedures for expanded NBS recruitment beginning July 2004. CCs choosing to expand recruitment need to investigate approvals that their local IRB may require.

3.7.1 Expanded Recruitment Plan

- Beginning July 2004, the plan for NBS recruitment expands to allow recruitment of non-AV NBS participants.

- This expanded recruitment plan is designed to:
  - Support CC efforts to meet NBS recruitment goals.
  - Continue to meet the criteria outlined in *Section 3.3 - Recruitment Plan:*
    - Minimize participant burden.
    - Avoid subjectively selecting participants (to avoid selection bias).
    - Ensure that the age and race/ethnic composition of the NBS sample is reasonably representative of the DM cohort.
    - Ensure that women known to have exclusion criteria (e.g., diabetes) do not receive an invitation letter.

3.7.2 NBS Study Materials for Expanded Recruitment

- The NBS protocol and study materials have been modified to allow expanded recruitment. The FHCRC IRB-approved revised materials can be found in the *NBS Manual* as follows:
  - See *NBS Manual, Appendix A – Protocol* for the *NBS Protocol, Version 3, July 2004*
  - See *NBS Manual, Appendix C – NBS Participant Materials* for *NBS Letter of Invitation, Version 4*[1]
  - See *NBS Manual, Appendix D – NBS Staff Materials* for the *NBS Telephone Screening Questionnaire/Script, Version 2, July 2004*
  - See *NBS Manual, Appendix B - NBS Consents* for *NBS Consent, Version 4, July 2004*[1]
  - See *NBS Manual, Appendix C - NBS Participant Materials* for *Study At A Glance, Version 4, July 2004*[1]
  - See *NBS Manual, Appendix C – NBS Participant Materials* for *Instructions for 24-hour Urine Collection, Version 3, July 2004*

  [1]Clinical centers conducting indirect calorimetry procedures at NBS Visit 2 (Chicago-Northwestern, Madison, and Pittsburgh) will use a different letter of invitation, consent, and Study at a Glance. For copies of the materials to be used only by CCs conducting indirect calorimetry procedures, refer to *NBS Manual, Section 10 – Indirect Calorimetry, Appendix for Section 10*.

- Clinical centers choosing to expand their NBS recruitment pool will use the revised materials and will have local IRB approval as necessary.

3.7.3 Supplemental Recruitment List for Expanded Recruitment

- In July 2004, the CCC provides each CC with a supplemental list of participants eligible for NBS by way of the *NBS Supplemental Recruitment Tracking* report (NBS002). The *NBS Supplemental Recruitment Tracking* report (NBS002) includes DM participants (Intervention and Control) who meet the following criteria:
  - Have a FFQ at baseline, Year 1, and at least one additional time point.
  - Have full-follow-up status.
  - Do not take insulin or oral hypoglycemic agents to manage diabetes.
  - Are not included on *NBS Recruitment Tracking* report (NBS001).

- For information about accessing the *NBS Supplemental Recruitment Tracking* report (NBS002) in WHILMA, refer to the *WHILMA Upgrade Notes for NBS Supplemental Recruitment Tracking Report* located in the Outlook Public Folders/All Public Folders/Nutri Biomarkers Study (dated 7/26/04).

3.7.4 Approaching Participants for Expanded Recruitment

- If a clinical center chooses to expand NBS recruitment, the NBS recruitment pool may be expanded in two ways:
  - Invite participants from the *NBS Recruitment Tracking* report (NBS001) whose Annual Visits (AVs) have already occurred.
  - Invite participants from the *NBS Supplemental Recruitment Tracking* report (NBS002).

- Invite only participants who are listed on the two NBS recruitment tracking reports (described immediately above). Participants whose names are not on the recruitment tracking reports cannot be invited to participate in NBS. The recruitment tracking reports pre-exclude WHI DM participants who do not meet the NBS pre-telephone screening eligibility criteria.

- To avoid any potential of selection bias (subjectively selecting participants), identify participants to invite using the procedures outlined below.

  When inviting participants from the *NBS Recruitment Tracking* report (NBS001):

  - Use the 'AV Target Date Between' parameter to choose an AV target date range to identify participants between X and XX dates. Invite all participants in the selected date range. Exception: do not invite participants whose strata have closed.
    - If you are still recruiting for the NBS reliability subsample (NBS Visits 3 and 4), choose only an AV target date range between **July and September 2004**. These participants will have Close-out visit target dates between January and March 2005. This will ensure that there is adequate time between NBS Visits 1 and 2 and the NBS reliability subsample visits.
    - If you have finished recruiting participants for the reliability subsample, but still need participants for NBS Visits 1 and 2, choose any date range to identify participants.

  - Choose a date range that allows mailing in small weekly batches; i.e., a weekly mailing of invitation letters should include only the number of participants that can reasonably be contacted within one week of the mailing.

When inviting participants from the *NBS Supplemental Recruitment Tracking* report (NBS002):

- Use the 'Close-out Target Date Between' parameter to choose a Close-out target date range to identify participants between X and XX dates. Invite all participants in the selected date range. Exception: do not invite participants whose strata have closed.
  - If you are still recruiting for the NBS reliability subsample (NBS Visits 3 and 4), choose only a Close-out visit target date range between **January and March 2005**. This will ensure that there is adequate time between NBS Visits 1 and 2 and the NBS reliability subsample visits.
  - If you have finished recruiting participants for the reliability subsample, but still need participants for NBS Visits 1 and 2, choose any date range to identify participants.

- Choose a date range that allows mailing in small weekly batches; i.e., a weekly mailing of invitation letters should include only the number of participants that can reasonably be contacted within one week of the mailing.

SECTION 4
SCREENING

4.0 Overview of Screening Process

- The NBS screening process determines the eligibility and interest of potential DM 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 a NBS Visit 1 for eligible and interested participants.

4.1 Screening Activities

4.1.1 Telephone Contact

- CCs telephone all participants who are mailed an invitation letter within one week of the NBS mailing. A NBS interviewer uses the Call Attempts Log in the *NBS Recruitment Tracking* report (NBS001), *NBS Supplemental Recruitment Tracking* report (NBS002), or a similar CC-designed sheet to record the dates when a participant is contacted by phone.
  - The CCC strongly recommends that a CC's Call Attempts Log contain the following information: date, time of day, day of the week, and a place for interviewer's comments.
  - For information about a sample Call Attempts Log, refer to *Nutritional Biomarkers Study-Recruitment Upgrade Notes* located in the Outlook Public Folders/All Public Folders/Nutri Biomarkers Study (dated 5/12/04).
- If the NBS 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 'Ineligible' on *Form 74 – Screening Result* (refer to *NBS Manual, Section 4.1.4 – Complete and Data Enter NBS Screening Result (Form 74).*

4.1.2 Determine Eligibility and Interest

- During the screening phone call, the NBS interviewer uses the *Telephone Screening Questionnaire/Script* to explain the NBS 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 *NBS Manual, Appendix D – NBS Staff Materials.*

4.1.3 Schedule NBS Visit 1

- If a participant is eligible and interested, the NBS interviewer schedules the NBS Visit 1 to coincide with the participant's WHI AV. Whenever possible, every effort should be made to avoid scheduling Dietary Change (Intervention) and Comparison (Control) women on the same day to avoid 'contamination' of the WHI DM Comparison group.

4.1.4 Complete and Data Enter NBS Screening Result *(Form 74)*

- The NBS interviewer completes a *Form 74* for all participants mailed the NBS invitation letter.

- The NBS interviewer records the participant's 'Final Screening Disposition' on *Form 74 – NBS Screening Result (Qx. 5 - Final Screening Disposition)*. The 'final disposition' represents the participant's status at the end of the recruitment/screening phone call:
  - 'Scheduled': Participant was eligible and interested in the NBS study and is scheduled for NBS Visit 1.
  - 'Ineligible': Participant was not eligible to participate in the NBS study.
  - 'Declined': Participant was not interested in participating in the NBS study.

- There are a few circumstances where the CC has the option to complete a *Form 74* for a participant without mailing the NBS invitation letter and conducting the *NBS Telephone Screening Questionnaire/Script*.

  - Circumstance #1:

    The CC knows with certainty that the participant will be ineligible per the eligibility questions on the *NBS Telephone Screening Questionnaire/Script* (e.g., the participant has moved away from the CC and would need to travel more than 200 miles for the NBS visit). In this case, the CC has the option to complete the *Form 74* for the participant without mailing the invitation letter and conducting the *NBS Telephone Screening Questionnaire/Script (*mark *Form 74, Qx. 5 – Final Disposition* as '2 – Ineligible').

  - Circumstance #2:

    For participants who have significant cognitive challenges (e.g., a diagnosis of dementia) or whose WHI participation may be jeopardized by receiving an NBS invitation letter, CCs have the option to complete the *Form 74* for the participant without mailing the invitation letter and conducting the *NBS Telephone Screening Questionnaire/Script* (mark *Form 74, Qx. 5 – Final Eligibility Disposition* as '2 – Ineligible'). 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.

- Key-enter *Form 74* only after the final result of the telephone screening call is known. For example, if a participant is unable to schedule her NBS Visit 1 appointment until she checks her family schedule, then *Form 74, Qx. 5 – Final Eligibility Disposition* would not be marked until the participant has been scheduled for NBS Visit 1.

- Key-enter the *Form 74 - NBS Screening Result* within 1 working day of completing the form. The CCC will use information from *Form 74* to monitor the DM strata goals for age and race/ethnicity.

- File the completed and data entered *Form 74* in the participant's chart (or special NBS notebook).

SECTION 5
NBS VISITS

5.0 Overview of NBS Visits

- There are two NBS Visits (NBS Visit 1 and NBS Visit 2): *Figures 5.1* and *5.3* – provide an overview of the specific tasks for NBS Visit 1 and NBS Visit 2. *Sections 5.1 - NBS Visit 1 (Day 1)* and *5.2 – NBS Visit 2 (Day 15)* describe the activities of the two visits in detail and follow the flow depicted in *Figure 5.1* and *Figure 5.3*.

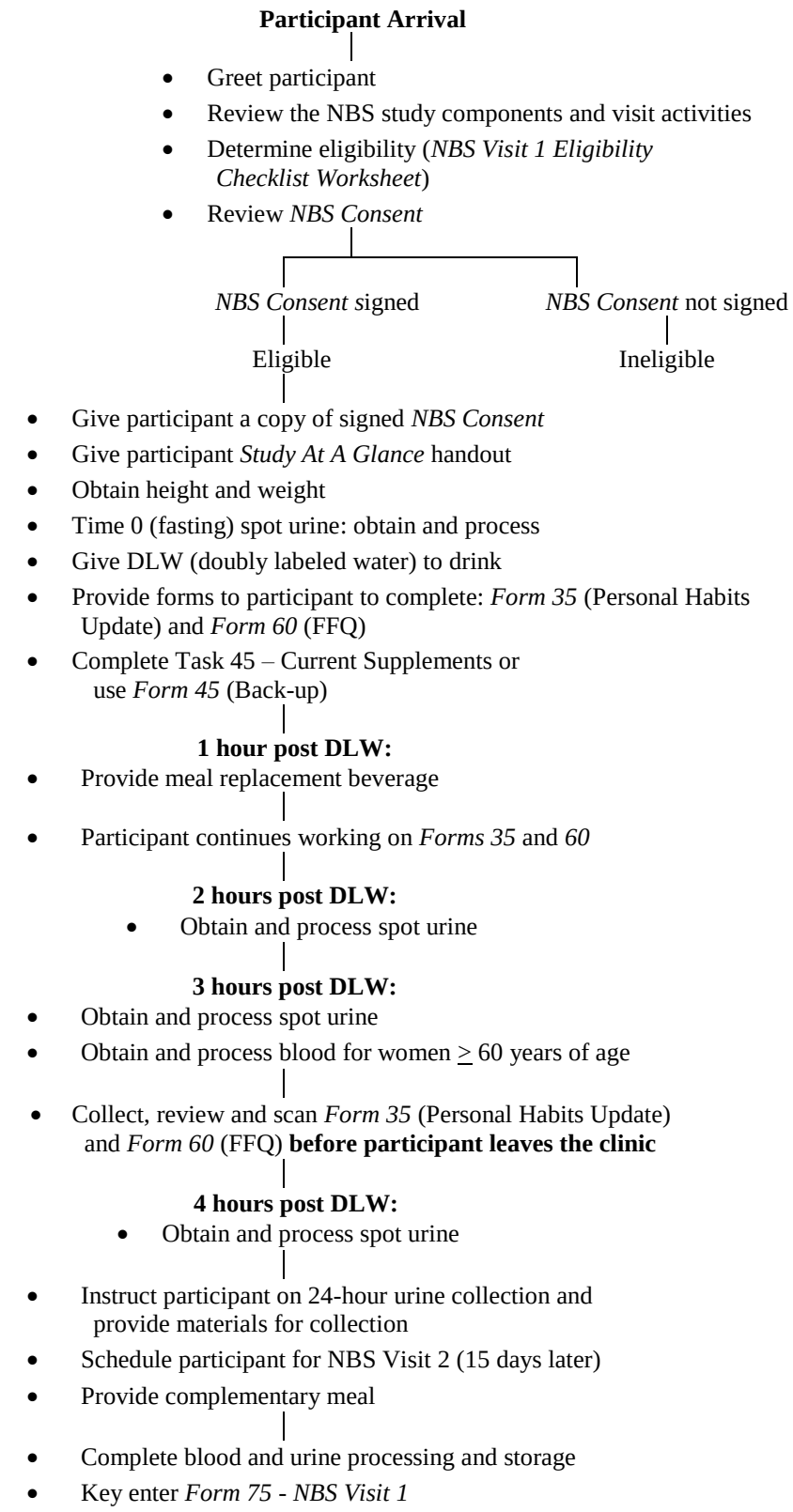
- In general, all visits contain similar activities in terms of preparation for the visit, greeting the participant, performing various visit activities, reviewing forms, providing the participant with appropriate materials before she leaves, scheduling the next visit, and data entry. Because the visits contain many different activities and the participant will most likely be seen by several different CC staff, it is critical to designate a NBS staff person to oversee the NBS visits (including pre- and post-visit activities).

5.1 **NBS Visit 1** (Day 1)

5.1.1 Overview of Activities

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

Figure 5.1
Overview of NBS Visit 1 (Day 1)



5.1.2 Integration of NBS Visit 1 and Annual Visit

- NBS Visit 1 occurs in conjunction with the WHI Annual Visit (AV). The NBS Visit 1 is integrated with the AV tasks so that all activities are completed as required and participant burden is minimized. *Figure 5.2. – Integration of NBS Visit 1 and Annual Visit Tasks* provides the estimated amount of time for each NBS task and shows how NBS Visit 1 and AV tasks could be integrated.

- It is important to BEGIN the participant's NBS Visit 1/AV with the NBS tasks and to MAINTAIN the sequence of NBS activities throughout the visit. This is important because:
  - The length of time a participant is in the clinic depends on when the NBS activities begin.
  - The sooner the NBS activities begin, the sooner the participant completes her clinic visit.
  - Having a NBS staff person be the <u>first person to see the participant</u> when she arrives, allows the 'visit eligibility' questions to be asked and the participant to begin NBS visit activities, if she remains eligible.
  - <u>The sequence of the NBS activities related to the doubly labeled water dosing and urine/blood collections is critical to the protocol and should not be changed.</u>

- The NBS protocol and AV tasks will take approximately 5 hours for participants to complete. DM-only participants may have some free time between tasks. When scheduling participants, encourage them to bring something to keep busy (e.g., crossword puzzles, a book, knitting, etc.). Women who are in Year 9 and/or other protocols (e.g., bone density protocol, ancillary studies) may need to stay longer than 5 hours.

- While the sequence of the NBS activities may not be changed, the sequence of the AV tasks may be modified to meet CC needs. It is critical to keep track of the time for NBS 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 <u>≥</u> 60 years of age).

- It is also important to have a designated NBS staff person oversee the NBS/AV activities – pre and post visit. Responsibilities could include:
  - Checking the *Tasks Required at Follow-up Visit* report (WHIP 0144) to identify NBS participants <u>scheduled to complete</u> *Forms 35, 45,* or *60* as part of their AV visit.
  - Verifying that participants scheduled to complete *Forms 35, 45,* or *60* as part of their AV have completed the form(s). If a participant scheduled to complete *Forms 35, 45,* or *60* as part of their AV forgets to bring the completed form to her AV, ask her to complete the form before she leaves the CC.
  - Ensuring that all WHI forms required by the NBS protocol (i.e., *Forms 35, 45,* or *60*) are collected, reviewed, and scanned <u>before</u> the participant leaves the clinic.

Figure 5.2 – Integration of NBS Visit 1 and Annual Visit Tasks

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

	Estimated Times	**NBS Visit Tasks**	**DM Annual Visit Tasks***	**CaD & HT Annual Visit Tasks***	**Year 9 Annual Visit Tasks***
45 min	5 min	Reception			Bone Density sites only - Collect first morning urine sample
	5 min	Complete *NBS Visit 1 Eligibility Checklist Worksheet*			
	20 min	Complete *NBS Consent*			
	5 min	Obtain- height and weight			
	10 min	Time 0 - Fasting spot urine collection and processing			
~ 60 min	5 min	Time 0 - Doubly Labeled Water Dosing –participant sits still for 15 minutes - completes forms			
	5 min		Obtain general medical releases		
	20 min	*Current Supplements* (*Task 45)*			
	10 min	*Personal Habits Update* (*Form 35*)			
	20 min				*Current Medications* (*Form 44*)
	5 min	Time 1 - Meal replacement beverage consumed: 1 hour after DLW			
~ 60 min	30 min	*FFQ (Form 60*) – participant completes			
	20 min		Breast exam – optional		
	10 min	Time 2 – Spot urine collection and processing: 2 hours after DLW			

Figure 5.2 – Integration of NBS Visit 1 and Annual Visit Tasks (continued)

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

	Estimated Times	NBS Visit Tasks	DM Annual Visit Tasks*	CaD & HT Annual Visit Tasks*	Year 9 Annual Visit Tasks*
~ 60 min	15min				Functional status - sub sample grip strength, chair stand, and timed walk (25% over 65)
	5 min			HT - Complete HT management and safety interview (*Form 10*)	
	10 min				HT - Cognitive assessment for ppts 65+
	20 min		*Physical Measurements - Form 80* (other than height & weight) Resting pulse, blood pressure		
	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			
	20 min	Review and scan *Form 35* and *Form 60*. Clarify missing information with participant.	Review *Form 33* Complete *Form 33D*, as needed		
	10 min			CaD - Complete adherence to study pills	
	5 min			CaD - Complete management and safety interview (*Form 17*)	
	5 min			CaD - Dispense study pills	
	5 min		Exit interview		
	10 min	Time 4 – Spot urine collection and processing - 4 hours after DLW			

Figure 5.2 – Integration of NBS Visit 1 and Annual Visit Tasks (continued)

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

	Estimated Times	NBS Visit Tasks	DM Annual Visit Tasks*	CaD & HT Annual Visit Tasks*	Year 9 Annual Visit Tasks*
~ 15 min	10 min	Provide instructions and kit for 24-hour urine collection -			
	5 min	Confirm appt. for next visit			
		Provide complementary meal (e.g., box lunch)			
	5 min				Daily Life – 6% sub sample
	30 min				ECG
	30 min				Bone density sites only - bone density scan

5.1.3 Pre-NBS Visit 1 Activities

5.1.3.1 Supplies and Forms

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

Supplies:

- WHI Folder (for participant handouts)
- NBS label set
- Urine collection hat
- Doubly labeled water (DLW)
- Lavender (dry EDTA) blood collection tube (for participants ≥ 60 years of age)
- Corning cryovials, 5 mL
- 2.0 mL cryovials
- Disposable transfer pipettes
- Ziplock bag with pre-weighed tissue
- Meal replacement beverage (e.g., Boost, Ensure)
- 24-hour urine collection kit (*Instructions 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)
  - For participants who receive PABA, the 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).
    - 3 sealed PABA (B-vitamin) tablets
    - 2 [3-liter] urine collection bottles in each of which you place 2.0 gram (1.5 level measuring teaspoons) 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"

      * 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:

- *NBS Visit 1 Eligibility Checklist Worksheet*
- *NBS Consent*
- *Form 75 - NBS Visit 1*
- *Form 60 – Food Frequency Questionnaire*
- *Form 35 – Personal Habits Update*
- *Form 45 – Current Supplements (Backup)* - (have available if not directly key-entering supplement information into WHILMA using Task 45)
- *NBS Visit 2 (or Visit 4) PABA Worksheet for Staff*

5.1.3.2 Pre-Visit 1 Reminder Call

- A NBS staff person calls each participant one day prior to her NBS 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 NBS Visit 1 appointment.

- Remind the participant to refrain from eating any food or drinking any caffeine or calorie-containing beverages for **4 hours** prior to her clinic appointment time.
- Let participant know that during the 4-hour fast she may take all her regular medications 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 supplements to the visit (they will be documented in Task *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.

5.1.4 NBS Visit 1 Activities

5.1.4.1 Reception

- When a participant first arrives at the CC for her NBS Visit 1/AV, 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 NBS Lead-ops (or designee) that the participant is waiting.
- If a participant arrives at the clinic for her AV without having been reached for the *NBS Telephone Screening Questionnaire/Script* and inquires about the NBS study, the Receptionist should immediately notify the NBS-Lead-ops (or designee) that the participant is interested in NBS. The Lead-ops (or designee) checks to see if the participant is on the *NBS Recruitment Tracking* report (NBS001). If yes, the Lead-ops (or designee) conducts the *NBS Telephone Screening Questionnaire/Script* to determine interest and eligibility. If eligible, the Lead ops (or designee) uses the procedures outlined in the *NBS Manual, Section 4.1.3 – Schedule NBS Visit 1* and *Section 4.1.4 – Complete and Data Enter NBS Screening Result (Form 74).*

5.1.4.2 Eligibility

- Some of the NBS eligibility questions asked during the telephone screening call are repeated at NBS Visit 1 to ensure that the participant remains eligible. Begin the participant's visit by using the *NBS Visit 1 Eligibility Checklist Worksheet* to update a participant's continuing eligibility.
  - If a participant reports any change in the information she previously provided on the *NBS Telephone Screening Questionnaire/Script* that now make her ineligible (e.g., began taking insulin or oral hypoglycemics), mark her "Ineligible" on *Form 75 – NBS Visit 1*. Thank the participant and escort her to the WHI staff person to start the WHI AV tasks. File the *NBS Visit 1 Eligibility Checklist Worksheet* in the participant's chart.
  - If a participant reports a change that would make her 'temporarily ineligible' (e.g., traveled more than 200 miles in past 2 weeks) for NBS Visit 1 and she is not willing to reschedule her NBS Visit 1, mark her "Ineligible" on *Form 75 – NBS Visit 1*. Thank the participant and escort her to the WHI staff person to start the WHI AV tasks. File the *NBS Visit 1 Eligibility Checklist Worksheet* in the participant's chart.
  - If a participant reports a change that would make her 'temporarily ineligible' (e.g., traveled more than 200 miles in past 2 weeks) for NBS Visit 1, but she is willing to reschedule her NBS Visit 1, thank the participant, reschedule her NBS Visit 1 for another day and then escort her to the WHI staff person to start the WHI AV tasks. File the *NBS Visit 1 Eligibility Checklist Worksheet* in the participant's chart.
- If a participant remains eligible, mark "Eligible" on *Form 75 – NBS Visit 1* and begin the *NBS Consent* procedures.

5.1.4.3 *NBS Consent*

- Refer to *NBS Manual, Appendix B – NBS Consents* and the *Appendix for Section 10 – Indirect Calorimetry,* for a copy of the appropriate *NBS Consent.*
- Mailing the *NBS Consent* Before NBS Visit 1. The CCC recommends that the *NBS Consent* not be sent with the NBS invitation letter. It could be overwhelming to the participant to read the *NBS Consent* without having had the opportunity to discuss the NBS protocol with staff. If a CC wants to send the *NBS Consent* to interested participants, the CC could mail the consent to the participant after she has completed the NBS phone screening contact and is scheduled for NBS Visit 1.

- Reviewing the *NBS Consent*. Provide the participant with a copy of the *NBS 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 Nutritional Biomarkers Study (NBS) is voluntary and does not affect her participation in the WHI Dietary Study. She may withdraw from NBS 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 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 NBS procedures during Visit 1 and Visit 2 (e.g., height, weight, administration of DLW, spot urines and blood draws).
  - 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.
  - The potential of repeating the entire study about six months later to help us learn about the reliability of the measurements. Participants who repeat the entire study will also be asked to complete two 20-minute dietary phone interviews (24-hour recalls).

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

- Signing the *NBS Consent*:
  - Once the participant's questions have been answered, ask her to sign and date two copies of the *NBS Consent* in the appropriate places. Sign two copies of the form yourself, as a WHI representative, and witness and date the form. Give one copy of the *NBS Consent* to the participant and file the other copy in her file. Record on *Form 75 – NBS Visit 1* that the participant has signed the *NBS Consent.*
  - If a participant declines to sign the *NBS Consent*, do not continue further NBS activities. Record on *Form 75 – NBS Visit 1* that the participant did not sign the *NBS Consent*. Thank the participant, end the NBS visit and have the participant continue with her regularly scheduled AV activities.

- Provide a WHI Folder: Provide each participant with a WHI folder to hold the NBS study materials provided at NBS Visit 1 (i.e., copy of *NBS Consent, Study 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 NBS, a copy of *Study At A Glance* (copy available in *NBS Manual, Appendix C – NBS Participant Materials*). This handout provides a brief overview of the activities that each participant will do as part of the Nutritional Biomarkers Study.

5.1.4.5 Physical Measurements (height and weight)

- Collect the height and weight on the day of the NBS Visit. Use a measured height and weight (not an estimate). 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 NBS study.

- Record the height and weight in centimeters and kilograms, respectively, on *Form 75 - NBS Visit 1*. Staff can copy the participant's NBS height and weight information onto her AV *Form 80 – Physical Measures* (i.e., once the participant's height and weight have been measured for NBS, they do not need to be re-measured for her AV).

5.1.4.6 Spot Urine Collection – NBS Visit 1

5.1.4.6.1 General Information

- There are four spot urine samples collected at NBS 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 *NBS 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 NBS Visit 1.

- **Thoroughly 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 NBS Visit 1. The urine hat will need to be rinsed and thoroughly dried with a 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.

- **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 NBS label on the cryovial; transfer the urine to the 5 mL cryovial, cap and store within 10 minutes. Refer to *NBS 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 NBS 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 75- NBS Visit 1*.

- Use the procedures described in the *NBS 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 NBS 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 are respectively:
  A – Less than 65 kg
  B – 66 to 80 kg
  C – 81-105 kg
  D – Greater than or equal to 106kg

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 Helen Penor (hpenor@whi.org) at the CCC if the CC is running out of a specific-sized bottle.

- Record staff ID (staff administering DLW), the 7-digit dose weight of the bottle and the lot number of the bottle on *Form 75- NBS Visit 1*. The doses vary slightly, so it is critical to get the correct information recorded on the form.

- Give the participant a straw and ask her to drink all the DLW (about ½ cup). 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 75- NBS Visit 1*.

- After the participant drinks the contents of the DLW bottle, there is usually about half a gram of labeled water still left in the bottle. To guarantee that the participant has received the entire DLW tracer, fill the DLW dose bottle with 50 mL tap water and push the straw into the bottle. Replace the cap and gently mix the water around so that it captures all the labeled water on the cap, inside of the bottle, and the straw. Give the participant a second straw and ask her to drink the rinse water. Note: the amount of the rinse water (50 mL) does not need to be recorded on *Form 75 – NBS 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 CC 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 'NBS DLW Spillage Container Visit 1' label on the participant's NBS label set.

- Place the participant's 'NBS DLW Spillage Container Visit 1' label on the bag and date the bag. Record 'Yes' for DLW spillage on the *Form 75- NBS Visit 1 (Qx. 13.5)*.

- The same day, Fed-Ex the bagged tissue to Dr. Dale Schoeller at address below:

  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 Storage of DLW

- 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 a NBS participant to drink.

5.1.4.8 Provide WHI *Forms 35* & *60* and Complete Task 45

- Give the participant *Form 35 (Personal Habits Update)* and *Form 60 (FFQ)* to complete. Ask her to complete these forms while she is waiting between visit activities. Let the participant know that a staff person will review both forms with her before the end of her visit.

- Complete *Task 45 - Current Supplements*
  - The purpose of collecting an inventory of current supplements at NBS Visit 1 is to document the supplements a participant takes that could influence the levels of vitamins and minerals in her blood.
  - Enter each current vitamin and mineral supplement into WHILMA following the procedures described in *WHI Manuals, Vol.5 – Data System, Section 7.3.2 – Current Supplements*. Remind the participant to report only current supplements taken at least once a week. The supplement inventory program will offer specific prompts for entering the vitamin formulations when needed. *Use Form 45 – Current Supplements (Backup)* for completing the supplements inventory if the computer is not available.
  - If the participant has forgotten to bring her supplements, arrange to have her bring them to NBS Visit 2 or call her at home to follow-up.
  - If a participant is completing *Task 45 - Current Supplements* as part of her AV, record '3 – Annual' in the "Visit Type" field on the form and indicate the appropriate year. If a participant is not completing *Task 45* as part of her AV visit, record as '4 – Non Routine' in the "Visit Type" field.

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, etc.) or other beverage of choice. Record "Yes" on *Form 75, Qx. 14.1 – 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 *NBS 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 NBS Visit 1.

- Record staff ID (for staff collecting the urine) and time of collection on *Form 75 – NBS Visit 1*.
- If a participant has problems with the void, follow the procedures for handling collection problems, described in the *NBS 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 75 – NBS Visit 1,* (*Qx. 18 - 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 75-NBS Visit 1* that the participant was unable to provide a Time X urine specimen. Follow the procedures outlined in the *NBS Manual, Section 8.2.1 – Notes on NBS Forms* to notify 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 75 – NBS Visit 1, (Qx. 18 - 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 *NBS 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 NBS Visit 1.
- Record staff ID (staff collecting the urine) and time of collection on *Form 75 - NBS Visit 1*.
- If a participant has problems with the void, follow the procedures for handling collection problems, described in the *NBS 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 75 - NBS Visit 1*. Use standard WHI blood handling and processing procedures described in the *NBS 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 75 –NBS Visit 1* next to the participant's member ID label – "Participant is under 60". This will help NBS 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 *NBS Manual, Section 7.4.4 – Blood Collection Problems*.

5.1.4.13 Collect and Review *Form 35* and *Form 60*

- *Form 35 (Personal Habits Update)* and *Form 60 (FFQ)* are to be completed, reviewed and scanned before the participant leaves the clinic. Whenever possible, use staff blinded to DM assignment to review the forms.

- Make sure that there is a WHI participant bar code label in the designated space on the front page of each completed *Form 35* and *Form 60*. (Refer to *WHI Manuals, Vol. 3 – Forms* for specific instructions regarding the items on each form).

- If a participant is completing a *Form 35* (Personal Habits Update) *or Form 60* (FFQ) as part of their AV, record '3 – Annual' in the "Visit Type" field on the form and indicate the appropriate year. For participants who are not completing a *Form 35* or *Form 60* as part of their AV visit, record '4 – Non Routine' in the "Visit Type" field on the form.

  a) Form 35 – Personal Habits Update
  - Information about the participant's physical activity will be captured using *Form 35 (Personal Habits Update*).

  b) Form 60 – Food Frequency Questionnaire
  - Information about the participant's dietary intake will be captured using *Form 60 (FFQ)*.
  - 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 *WHI Manuals, Vol. 3 – Forms* for more details about reviewing and editing the *FFQ*.

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 *NBS 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 NBS Visit 1.

- Record staff ID (staff collecting the urine) and time of collection on *Form 75 - NBS Visit 1*.

- If a participant has problems with the void, follow the procedures for handling collection problems, described in the *NBS 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 NBS 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 75 - NBS Visit 1 (Qx. 18 - Other Beverages Consumed*)

- If a participant does not drink any other beverages between hours 2-4 after the DLW, record '0' for *Qx. 18.1* (*Beverage 1 Amount*) and *Qx. 18*.2 (*Beverage 2 Amount*) on *Form 75 - NBS Visit1*.

5.1.4.16 Explain and Provide Materials for 24-hour Urine Collection

- Near the end of NBS 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 NBS Visit 2 (Day 14) and bring it back to her NBS 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 NBS Visit 2 on Day 15. However, for participant convenience, NBS 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 *NBS Manual, Appendix C – NBS 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 provide urine collection bottles that do not have boric acid inside.
      - **Record in the "Notes" section of *Form 75 – NBS Visit 1* (or *Form 77 – NBS Visit 3*) that the participant was not given the PABA (B-vitamin) tablets because she has had past hypersensitivity to PABA**. Follow the procedures outlined in *NBS Manual, Section 8.2.1 – Notes on NBS Forms* to notify the CCC
    - 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 NBS Visit 2.

- Attach a WHI participant member ID to a *NBS Visit 2 (or Visit 4) PABA Worksheet for Staff* and mark the appropriate response for Question 1 (*Did the 24-hour urine kit contain the 3 PABA (B-vitamin) tablets?*). Place the *NBS Visit 2 (or Visit 4) PABA Worksheet for Staff* in the participant's chart (or special NBS notebook) so that it will available for use at NBS Visit 2 (or Visit 4).

- 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). For participants who receive PABA, substitute 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).
  - Two 3 liter urine collection bottles. For participants who receive PABA, substitute 2 [3-liter] containers that have 2.0 gram (1.5 level measuring teaspoons) 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"
  - One urine collection hat
  - One plastic funnel
  - One large safety pin
  - Two gel ice packs
  - One plastic carrying bag
  - For participants who receive PABA, the kit additionally contains 3 sealed PABA (B-vitamin) tablets.
- Inform the participant that a NBS 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 NBS Visit 2. For participants who receive PABA (B-vitamin) tablets, explain that she will also be asked to bring the PABA tablet packaging with her to NBS Visit 2.

5.1.4.17 Confirm Appointment for NBS Visit 2

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

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

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).

5.1.4.19 Check Visit 1 Completeness

- Use the Completeness Checklist on the last page of *Form 75- NBS Visit 1* to check that all NBS Visit 1 activities have been completed. Complete any remaining tasks, if required. The staff member who reviews *Form 75 – NBS Visit 1* and checks completion of NBS Visit 1 tasks, designates completion by recording their Staff ID on *Form 75- NBS Visit 1 (Qx. 20 – Visit 1 Completeness Staff ID).*

5.1.5 Post-NBS Visit 1 Activities

5.1.5.1 Follow-up on Missing Forms

- CCs should have a data back-up plan if problems arise with collection of *Form 35* (Personal Habits Update*)* and *Form 60* (FFQ). If a participant leaves the clinic and NBS staff discover that *Form 35* –or *Form 60* are missing or have incomplete data, assign a staff person to follow-up with the participant within **1-2 days** of NBS Visit 1.

5.1.5.2 Data Entry

- Key-enter *Form 75 - NBS Visit 1* for all participants within 1-2 days of NBS Visit 1. File the completed and key-entered *Form 75 - NBS Visit 1* in the participant's chart (or special NBS notebook).

5.2 **NBS Visit 2** (Day 15)

5.2.1 Overview of Activities

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

Figure 5.3
Overview of NBS Visit 2 (Day 15)

Participant Arrival

- Greet participant
- Complete *NBS Visit 2 Participant Update Worksheet*
- Receive 24-hour urine collection
  - Obtain weight
  - Obtain and process Time 0 (fasting) spot urine sample
  - Obtain and process Time 0 (fasting) blood draw
  - Provide snack/meal
  - Obtain and process Time 1 spot urine (1-hour post fasting urine)
- Discuss repeat of NBS protocol in about 6 months. Complete *Consent for Future Contact and Continued Participation in this Study*
  - Schedule NBS 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 NBS protocol
  - Key enter *Form 76 - NBS Visit 2* and the information from the *NBS Visit 2 (or Visit 4) PABA Worksheet for Staff*

5.2.2 Pre-NBS Visit 2 Activities

5.2.2.1 Supplies and Forms

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

Supplies:

- NBS label set (**must be** the remainder of the label set started at NBS Visit 1 for the specific participant)
- Royal blue and lavender (dry EDTA) blood collection tubes
- Corning cyrovials, 5mL
- 2.0 mL cyrovials
- Graduated cylinder to measure 24-hour urine
- Urine collection hat
- Disposable transfer pipettes
- Snack

Forms:

- *NBS Visit 2 Participant Update Worksheet*
- *Form 76 - NBS Visit 2*
- *NBS Consent for Future Contact and Continued Participation in this Study*
- *NBS Visit 2 (or Visit 4) PABA Worksheet for Staff* (should be in participant's file from Visit 1 [or Visit 3])

5.2.2.2 Pre-Visit 2 Reminder Call

- **Two days prior** to the participant's scheduled NBS Visit 2, call the participant. Remind her to start the 24-hour urine collection by collecting all of her urine beginning at 8 AM the next day using the specially provided urine collection kit.

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 NBS 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. If the urine kit contains the PABA (B-vitamin) tablets:
       She should take one 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 during 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.

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 NBS 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 *NBS Manual*, *Appendix C – NBS 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 NBS Visit 2.
    If the participant received PABA (B-vitamin) tablets, she is to bring back the PABA tablet packaging with her to NBS Visit 2.

5.2.2.2.2 Confirm Appointment and Fasting Time

- Confirm the participant's NBS 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 NBS Visit 1.**
- Remind the participant to:
  - Refrain from eating any food for **6 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 NBS Visit 2.
  - Take all her regular medications with water on the day of NBS 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.
- Ask the participant if she has questions and provide information as needed.

5.2.3 NBS Visit 2 Activities

5.2.3.1 Reception – Welcome

- When the participant first arrives for NBS 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 NBS Lead-ops (or designee) that the participant is waiting.

5.2.3.2 Complete *NBS Visit 2 Participant Update Worksheet*

- Complete *NBS Visit 2 Participant Update Worksheet* to monitor and note potential changes that might influence the spot urine samples collected at NBS Visit 2. None of the questions on this worksheet will make a participant ineligible (i.e., travel, use of IV fluids, etc.). The NBS Lead-ops (or designee) reviews the *NBS Visit 2 Participant Update Worksheet* and takes the appropriate actions outlined below.
  - If a participant marks 'No' to questions 1-4 on the *NBS Visit 2 Participant Update Worksheet,* NBS staff should:
    - Record their staff ID on *Form 76 – NBS Visit 2, Qx. 5 – Staff ID to Indicate Completion of the Visit 2 Participant Update Worksheet.*
    - Begin NBS Visit 2 activities.
  - If a participant marks 'Yes ' to question 1, 2 or 3 on the *NBS Visit 2 Participant Update Worksheet,* NBS staff should:
    - Note the participant's response on the line next to the appropriate question.
    - Record their staff ID on *Form 76 – NBS Visit 2, Qx. 5 – Staff ID to Indicate Completion of the Visit 2 Participant Update Worksheet.*
    - Begin NBS Visit 2 activities.
    - At the end of NBS Visit 2, follow the procedures outlined in the *NBS Manual*, *Section 8.2.1 – Notes on NBS Forms* to notify the CCC.
  - If a participant marks 'No 'to questions 1, 2 and 3, but 'Yes' to question 4 on the *NBS Visit 2 Participant Update Worksheet* (indicating that she has not fasted for 6 hours), NBS staff should:
    - Record their staff ID on *Form 76 – NBS Visit 2, Qx. 5 – Staff ID to Indicate Completion of the Visit 2 Participant Update Worksheet.*
    - Refer to the procedures in the *NBS Manual*, *Section 5.2.3.6.1-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 NBS Visit 2 activities.

5.2.3.3 Receive 24-hour Urine Collection

- Follow procedures outlined in the *NBS 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 *NBS Visit 2 (or Visit 4) PABA Worksheet for Staff*.

  Handling 24-hour Urine Collection Problems:
  - If a participant arrives at the CC with an incomplete or contaminated 24-hour urine collection, the Lead-ops (or designee) follows the procedures outlined in the *NBS Manual, Section 8.2.1 – Notes on NBS Forms* to notify the CCC.
  - If a participant arrives at the CC 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 NBS 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 NBS Visit 2 activities.

- **Participant Refuses to Collect 24-hour Urine**: If a participant refuses to complete her 24-hour urine collection, the NBS Lead-ops (or designee) continues with the remaining NBS Visit 2 activities and at the end of the visit, follows the procedures outlined in the *NBS Manual, Section 8.2.1 – Notes on NBS Forms* to notify the CCC.

5.2.3.4 Physical Measurements (weight)

- Measure the participant's weight in kilograms and record on *Form 76 - NBS Visit 2*.

5.2.3.5 Spot Urine Collection - NBS Visit 2

5.2.3.5.1 General Information

- There are two spot urine samples collected at NBS Visit 2: a fasting sample and a sample one hour later. Use the procedures described in the *NBS 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 NBS Visit 2.

- Use the guidelines provided in the *NBS Manual, Section 5.1.4.6.1 - General Information* to thoroughly dry collection devices and avoid leaving the urine sample open to evaporation.

5.2.3.5.2 Time 0 (Fasting) Urine Collection

- The Time 0 (fasting) spot urine sample needs to be collected, as soon as possible, after completing *NBS Visit 2 Participant Update Worksheet* and obtaining the participant's weight. Use the procedures described in the *NBS 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 NBS Visit 2.

- When the sample is collected, record staff ID (for staff collecting the urine) and time of collection on *Form 76 - NBS Visit 2*.

5.2.3.6 Fasting Blood

- A fasting blood sample is required for all participants at NBS Visit 2. Draw the participant's blood if she has been fasting for at least **6 hours**. Use standard WHI blood handling and processing procedures described in the *NBS Manual, Section 7.3.3.2.3 – Time 0 (Fasting) Blood Draw*.

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

5.2.3.6.1 Handling Non-Fasting Participants

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

5.2.3.7 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).

5.2.3.8 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 *NBS 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 NBS Visit 2.

- When sample is collected, record staff ID (for staff collecting the urine) and time of collection on *Form 76- NBS Visit 2.*

5.2.3.9 Invite Repeat of NBS - (Reliability Subsample)

- After the final spot urine, give the participant the *Consent for Future Contact and Continued Participation in this Study.* Refer to *NBS Manual, Appendix B – NBS 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 Nutritional Biomarkers 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 Nutritional Biomarkers Study in about six months" – it means that she is consenting to be part of the reliability subsample (110 women).

- Explain that by agreeing to repeat the Nutritional Biomarkers 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). She will also be asked to complete two telephone interviews about her food intake - the first about 3 weeks after the NBS Visit 2 and another around the time she repeats the NBS study in about 6 months. She will also be asked to take a B-vitamin (PABA) the day she collects her 24-hour urine.

- There should be at least two months between NBS Visit 2 and the first reliability subsample visit (NBS Visit 3). Whenever possible, schedule NBS Visit 3 to occur in conjunction with the participant's WHI Close-out visit (NBS Visit 3 may be on the same day or a few days before or after the Close-out visit). In many cases, this scheduling will support the appropriate timing between NBS Visits 1 and 2 and NBS Visits 3 and 4. On average, there will be approximately 6 months between the WHI AV and the WHI Close-out visit. However, if a participant's WHI Close-out visit is scheduled for less than two months after NBS Visit 2, please choose one of the following options:
  - Do not offer the participant an opportunity to participate in the reliability subsample.
  - Schedule the participant's WHI Close-out visit so that there are at least two months between NBS Visit 2 and her WHI Close-out visit (and NBS Visit 3), if this is manageable for your CC.

- Note: Each CC strives to recruit about 13 participants for the reliability subsample (Tucson and Memphis strive to recruit about 7 participants). 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 NBS 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 CCs know if they need to recruit more or fewer than 13 participants for the reliability subsample.

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

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

- **Thank You**. Thank the participant for her participation in the NBS study. If the participant has agreed to be part of the reliability subsample, let her know that NBS staff will contact her within the next 6 months to conduct two 24-hour recalls.

5.2.4 Post-NBS Visit 2 Activities

5.2.4.1 Data Entry

- Key-enter *Form 76-NBS Visit 2* for all participants within 1-2 days of NBS Visit 2; include the information from *NBS Visit 2 (or Visit 4) PABA Worksheet for Staff*. File *Form 76* and *NBS Visit 2 (or Visit 4) PABA Worksheet for Staff* in the participant's chart (or special NBS notebook).

5.2.4.2 24-hour Recalls

- For participants who agree to be part of the reliability subsample, the CCC will send each woman a portion size booklet and a personal letter advising her that staff from the WHI Coordinating Center in Seattle will contact her approximately 3 weeks after her *NBS Visit 2* for the first 24-hour recall. A second 24-hour recall will be completed around the time that the participant repeats the NBS protocol about 6 months later.

SECTION 6
NBS RELIABILITY SUBSAMPLE
(Information to be added)

SECTION 7
BLOOD AND URINE COLLECTION, PROCESSING, AND SHIPMENT

Introduction:

This section of the manual for the WHI Nutritional Biomarkers Study (NBS) describes procedures for collecting, processing, and shipping blood and urine samples for the WHI NBS.

The NBS 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:

NBS Visit 1

During NBS Visit 1 (or NBS 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 NBS Visit 2.

24-hour Urine Collection

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

NBS Visit 2

During NBS Visit 2 (or NBS 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:

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

CC staff ship specified serum, plasma, and urine samples to McKesson BioServices in Rockville, MD. McKesson 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). Two CCs (Pittsburgh and Stanford) will prepare all of the NBS QC samples.

7.1 General Guidelines

7.1.1 Safety Procedures - Precautions for Handling Blood

Refer to *WHI Manuals: Volume 2 – Procedures; Section 11.1.1 - Safety Procedures - Precautions for Handling Blood*).

7.1.2 Safety Procedures - Precautions for Handling Urine

Follow relevant safety precautions described in *WHI Manuals: Volume 2 – Procedures; Section 11.1.1 - Safety Procedures - 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 and Certification

In general, refer to *WHI Manuals: Volume 2 – Procedures; Section 11.1.2 – Training and Certification*.

NBS specifics:

- A WHI CC staff person may draw, process and ship the blood and urine specimens for the NBS after completing the NBS training activities and being certified for specified WHI tasks (refer to *NBS Manual Section 2.2.2 – Non-Lead NBS Staff*).

7.1.4 Facilities

Refer to *WHI Manuals: Volume 2 – Procedures; Section 11.1.3 –Facilities.*

7.1.5 Supplies

Refer to *NBS Manual, Section 2.4.4 – Guidelines for Ordering Supplies* for information about supplies for blood and urine collection, processing and shipment.

7.1.6 Labels

The CCC provides the CCs with NBS label sets. **There is one NBS label set per participant**. This means that all the blood and urine specimens collected from a participant at NBS Visit 1 and NBS Visit 2 have the same specimen identification number. Each label set contains 42 labels, all of which are needed for the NBS Visit 1 and NBS Visit 2 forms, sample tubes, and sample aliquots. Refer to *Figure 7.1 NBS Label Set (Visits 1 and 2)*.

- There are 18 labels for NBS Visit 1: 10 labels with bar codes to be used for the form, DLW spillage, blood tube, blood and urine aliquots, plus 8 labels without bar codes used as descriptors or blank labels.
- There are 24 labels for NBS Visit 2: 19 labels with bar codes to be used for the form, indirect calorimetry (for CCs conducting indirect calorimetry), blood tube, blood and urine aliquots, plus 5 labels without bar codes used as descriptors or blank labels.

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

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

KEY POINTS (about the NBS label set):

- Each label set has an identification number.
- Label all the specimens collected at NBS Visits 1 and 2 with this identification number using the appropriate bar-coded NBS label.
- Label *Form 75 – NBS Visit 1* and *Form 76 – NBS Visit 2* with this identification number using the appropriate bar-coded NBS label. There is only one label for each of the NBS Visit forms. During data entry, there will be a check on key-entry of *Form 76 – NBS Visit 2* to ensure that that the label set identification number matches the one entered on *Form 75 – NBS 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 NBS 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 NBS label set apply to NBS Visits 3 and 4.

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

Figure 7. 1 NBS Label Set (Visits 1 and 2)

**********	NBS Label Set 920003 M	**********
NBS VISIT 1 DO NOT USE	[BAR CODE] 920003 M FORM 75 NBS Visit 1	[BAR CODE] 920003 M NBS DLW SPILLAGE Container Visit 1
NBS Visit 1 Spot Urine DO NOT USE	[BAR CODE] 920003-30 NBS URINE Aliquot Visit 1 Time 0	[BAR CODE] 920003-32 NBS URINE Aliquot Visit 1 Time 2
	[BAR CODE] 920003-33 NBS URINE Aliquot Visit 1 Time 3	[BAR CODE] 920003-34 NBS URINE Aliquot Visit 1 Time 4
NBS Visit 1 60+ Blood DO NOT USE		[BAR CODE] 920003 M NBS BLOOD Tube Visit 1
[BAR CODE] 920003-40 Lavender Aliquot NBS Plasma Visit 1	[BAR CODE] 920003-41 Lavender Aliquot NBS Plasma Visit 1	[BAR CODE] 920003-42 Lavender Aliquot NBS Plasma Visit 1

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

NBS VISIT 2 DO NOT USE	[BAR CODE] 920003 M FORM 76 NBS Visit 2	[BAR CODE] 920003 M NBS Indirect Calori- metry
NBS Visit 2 Spot Urine DO NOT USE	[BAR CODE] 920003-50 NBS URINE Aliquot Visit 2 Time 0	[BAR CODE] 920003-51 NBS URINE Aliquot Visit 2 Time 1
NBS Visit 2 Fasting Blood DO NOT USE	[BAR CODE] 920003 M NBS BLOOD Tube Visit 2	[BAR CODE] 920003 M NBS BLOOD Tube Visit 2
[BAR CODE] 920003 M NBS BLOOD Tube Visit 2	[BAR CODE] 920003 M NBS BLOOD Tube Visit 2	[BAR CODE] 920003-02 Royal Blue Aliquot NBS Serum Visit 2
[BAR CODE] 920003-03 Royal Blue Aliquot NBS Serum Visit 2	[BAR CODE] 920003-04 Royal Blue Aliquot NBS Serum Visit 2	[BAR CODE] 920003-05 Royal Blue Aliquot NBS Serum Visit 2
[BAR CODE] 920003-10 Lavender Aliquot NBS Plasma Visit 2	[BAR CODE] 920003-11 Lavender Aliquot NBS Plasma Visit 2	[BAR CODE] 920003-12 Lavender Aliquot NBS Plasma Visit 2
NBS Visit 2 24-hour Urine DO NOT USE		[BAR CODE] 920003 M NBS URINE Tube Visit 2 24-hour
[BAR CODE] 920003-60 NBS URINE Aliquot Visit 2 24-hour	[BAR CODE] 920003-61 NBS URINE Aliquot Visit 2 24-hour	[BAR CODE] 920003-62 NBS URINE Aliquot Visit 2 24-hour

7.1.7 Quality Control (QC) for Laboratory Analysis

Laboratory quality control will be assessed on a 5% subsample of NBS specimens, i.e., specimens from 30 participants NBS-wide for NBS Visits 1 and 2 and from 6 participants NBS-wide for NBS Visits 3 and 4. The analytic laboratories will receive the QC samples as blinded duplicate aliquots.

For efficiency, two CCs will prepare the NBS QC samples. Each of the two QC CCs will prepare samples from 15 participants at NBS Visits 1 and 2 and from 3 participants at NBS Visits 3 and 4. The QC participants need to remain the same for both NBS Visits (i.e., participants identified for QC at NBS Visit 1 will continue to be part of the QC at NBS Visit 2).

Blood QC.

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

Urine QC.

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

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

7.2 NBS Visit 1 (or NBS 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 two QC CCs)
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 NBS Visit 1 (or NBS Visit 3) - QC CCs only

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

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

- For NBS Visit 1, each of the two QC CCs will collect QC aliquots from 15 participants (for a total of 30 participant-sets NBS-wide).
- For NBS Visit 3, each of the two QC CCs will collect QC aliquots from 3 participants (for a total of 6 participant-sets NBS-wide).

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

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

Urine. For the spot urine collections at NBS Visit 1 (or NBS Visit 3), collect two additional 4 mL aliquots per NBS 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 NBS Visit 1 (or NBS Visit 3) (Day 1)

7.2.2.1 Before the Participant Arrives

1. Obtain a *Form 75 – NBS Visit 1* (or *Form 77 – NBS Visit 3*).
2. Obtain one NBS label set for the participant. (For QC CCs, obtain 3 label sets for each QC participant.)
3. Insert the *Form 75 – NBS Visit 1* and the NBS label set into the participant's file (or *Form 77 – NBS Visit 3* for the reliability subsample). Write the participant's name on one of the blank labels in the NBS label set or place the participant's WHI member ID label on the back of the NBS label set so that it can easily be identified as belonging to that specific participant.
4. Arrange the lavender dry EDTA tube in a test tube rack (for participants 60 years of age and older). Arrange corresponding 2 mL cryovials in rack.
5. Arrange the 5 mL cryovials in a rack for the spot urine aliquots.

7.2.2.2 During the Visit (Overview)

1. Verify that the participant is eligible and has signed the *NBS Consent* by reviewing *Form 75 – NBS Visit 1* (*or Form 77 – NBS Visit 3*), question numbers 5, 6, and 7.
2. Apply the NBS form label with the identification number on *Form 75 – NBS Visit 1* (*or Form 77 – NBS Visit 3*). For participants in the QC subsample, label the appropriate section of the NBS form.
3. Review with the participant the blood and urine collection procedures for NBS Visit 1 (or NBS Visit 3).
4. Collect the spot urine samples for Time 0 (*NBS Manual Section 7.2.2.2.3*), Time 2 (*NBS Manual, Section 7.2.2.2.4*), and Time 3 (*NBS 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 (*NBS Manual, 7.2.2.2.6*).
6. Collect the spot urine sample for Time 4 (*NBS 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 (*NBS 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 75 – NBS Visit 1* (or *Form 77 – NBS Visit 3*). Subsequent collections may be collected at their scheduled times.

Example 1: NBS 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 *Form 75 – NBS Visit 1* (or *Form 77 – NBS 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: NBS Visit 1. A woman ingests the DLW at 9:00 AM and she needs to void 90 minutes later (10:30 AM). On *Form 75 – NBS Visit 1* (or *Form 77 – NBS 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 NBS 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 by thoroughly dried.**

Refer to *NBS 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 *NBS Consent* by reviewing *Form 75 – NBS Visit 1* (*or Form 77 – NBS Visit 3*), question numbers 5, 6, and 7.

Collect the **fasting spot urine** sample by following steps in the *NBS Manual, Section 7.2.2.2.2 - DLW Spot Urine Collection Steps*. Note the exact collection time on *Form 75 – NBS Visit 1* (or *Form 77 – NBS 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 *NBS 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 75 – NBS Visit 1* (or *Form 77 – NBS 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 *NBS 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 75 – NBS Visit 1* (or *Form 77 – NBS 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 75 – NBS Visit 1* (or *Form 77 – NBS Visit 3*). **Coordinate the time to occur just after the 3-hour spot urine collection**. Note the exact time on the NBS Visit form.

1. Follow general preparation for the participant (refer to *NBS 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 (*NBS 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 *NBS Manual Section 7.4.4.7 Blood and Urine Collection, Processing, and Shipping; 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 75 - NBS Visit 1*. Follow the procedures outlined in the *NBS Manual, Section 8.2.1 – Notes on NBS 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 *NBS 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 *NBS 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 75 – NBS Visit 1* (or *Form 77 – NBS Visit 3*).

7.2.2.2.8 Instructions for 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 1.5 level measuring teaspoons 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 base, 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 preservative inside bottle" and a second sticker that reads "Remember…take the PABA (B-vitamin) tablets" (stickers supplied by the CCC).

General instructions for the 24-hour urine collection

- Refer to *NBS 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.

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

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

7.3.1.1 Sampling and Aliquot Schedule

TIME	SAMPLE	ALIQUOT	QC (for the two QC CCs)
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 NBS 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 NBS aliquots needed for QC. QC samples will be pulled from WHI banked QC samples.
Received from participant	24-hour urine collection	Three, 1.8 mL (2 mL cryovial)	Six, 1.8 mL (2 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 NBS Visit 2 (or NBS Visit 4) - QC CCs only

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

The two QC CCs will collect QC aliquots by convenience sampling until the specified number of participants have had QC specimens collected. For an NBS QC participant at NBS Visit 2 (or NBS 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 NBS Visit 2, each of the two QC CCs will collect QC aliquots from 15 participants (for a total of 30 participants NBS-wide).
- For NBS Visit 4, each of the two QC CCs will collect QC aliquots from 3 participants (for a total of 6 participants NBS-wide).

Labels. For each QC participant, use two additional NBS participant label sets for the NBS Visit forms and QC aliquots. For NBS Visit 2 (or NBS Visit 4) carry forward use of the label sets stored with the participant chart from NBS Visit 1 (or NBS Visit 3).

Blood. Staff do not need to collect NBS QC serum or plasma aliquots at NBS Visit 2 (or NBS Visit 4). Quality control of blood sample analyses for NBS Visit 2 will be done using 30 blind duplicate pairs from banked WHI QC samples. For the 20% reliability subsample at NBS Visit 4, two pairs of banked WHI blind duplicate samples will be analyzed for QC.

Urine:

- For spot urine collections at NBS Visit 2 (or NBS Visit 4), collect two additional 4 mL aliquots per NBS 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, collect six additional 1.8 mL aliquots for each NBS QC participant. Label three of these additional aliquots from one set of the two participant label sets reserved for QC and label the second three additional aliquots from the remaining participant label set put aside.

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

Refer to *NBS Manual, Section 5.2.2.2 - Pre-Visit 2 Reminder Call* and *Section 5.222.1 – Review Instructions for 24-hour Urine Collection (Day 14)*

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

7.3.3.1 Before the Participant Arrives

1. Obtain *Form 76 – NBS Visit 2* (or *Form 78 – NBS Visit 4*).
2. Apply the NBS form label with the identification number on the NBS Visit 2 form.
3. Insert the labeled *Form 76 – NBS Visit 2* and the label set into the participant's file (or *Form 78 – NBS Visit 4*).
4. Arrange the three blue serum tubes and one dry lavender EDTA tubes in a test tube rack.
5. Arrange the two 5 mL urine cryovial tubes in a rack.

7.3.3.2 During the Visit (Overview)

1. Verify that the *NBS Visit 2 Participant Update Worksheet* has been completed and that before the blood draw is taken that the participant has been fasting for six hours.
2. Review with the participant the blood and urine visit procedures for NBS Visit 2 (or NBS Visit 4).
3. Receive the 24-hour urine collection from participant (*NBS Manual, Section 7.3.3.2.1*).
4. Collect the Time 0 (fasting) spot urine sample (*NBS Manual, Section 7.3.3.2.2*).
5. Draw the Time 0 (fasting) blood sample (*NBS Manual, Section 7.3.3.2.3*).
6. Collect the Time 1 (1-hour) spot urine sample (*NBS Manual, Section 7.3.3.2.4*).
7. Process the 24-hour urine collection (*NBS Manual, Section 7.3.3.2.5*).

7.3.3.2.1 Receive the 24-hour Urine Collection From Participant

1. Mark the participant's name on the collection container(s) using a Sharpie® pen or write her name on a blank label from the participant's label set and 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 *NBS 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 WHI participant member ID label to *Record Sheet for 24-hour Urine Collection* (last page - *Instructions for 24-hour Urine Collection*, given at NBS 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 *NBS Manual, Section 8.2.1 - Notes on NBS Forms* to notify the CCC.
7. Store the *Record Sheet for 24-hour Urine Collection* in the participant's file.
8. Use the *NBS Visit 2 (or Visit 4) PABA Worksheet for Staff* to record the following information about the PABA (B-vitamin) tablets:
   a. Qx. 1: *Did the 24-hour urine kit contain the 3 PABA (B-vitamin) tablets?* ('Yes' or 'No'). This question should have been marked by staff at the participant's NBS Visit 1 (or Visit 3). If not, verify whether or not the participant's urine kit contained the 3 PABA (B-vitamin) tablets and mark the appropriate response.
   b. Qx. 2 (only if 'Yes' to Qx. 1): *How many PABA (B-vitamin) tablets did the participant take?* If the participant does not have the packaging with her, ask her how many of the PABA (B-vitamin) tablets that she took ('0', '1', '2', '3', or 'Don't know').
   c. Qx. 3 (only if 'Yes' to Qx. 1): *Did the participant take any acetaminophen (for example Tylenol®) during the urine collection?* ('Yes', 'No' or 'Don't know').
   d. Qx. 4 (only if 'Yes' to Qx. 1): *Did the participant take any vitamins, other than the PABA (B-vitamin) tablets during the urine collection?* ('Yes', 'No' or 'Don't know').
9. Staple the *NBS Visit 2 (or Visit 4) PABA Worksheet for Staff* to *Form 76 – NBS Visit 2* (or *Form 78 – NBS Visit 4).*

7.3.3.2.2 Time 0 (Fasting) Urine Collection

Collect the **fasting spot urine** sample following the steps outlined in *NBS Manual, Section 7.2.2.2.2 – DLW Spot Urine Collection Steps*. Note the exact time on *Form 76 – NBS Visit 2* (or *Form 78 – NBS 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 six hours.
2. Follow general preparation for the participant (refer to *NBS 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 *NBS 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 *NBS Manual, Section, 7.4.4.7 Deficient Serum and Plasma Samples*. The remainder of NBS 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 *NBS 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 76 – NBS Visit 2* (or *Form 78 – NBS Visit 4*).

7.3.3.2.5 24-hour Urine Collection Processing

The 24-hour urine collection may be processed between the Time 0 and Time 1 Urine Collections if time is available, or after the two spot urine collections are completed. Follow these steps for processing the 24-hour urine collection:

1. Measure the total volume of urine collected using the graduations on the side of the urine collection container. Estimate to the nearest 25 mL graduation line, which is the smallest graduation on the bottle. If needed, use an appropriate graduated cylinder.
2. Document the total volume on *Form 76 – NBS Visit, Qx. 11.2* (or *Form 78 – NBS 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. If the participant brings in more than one 3-liter container, mix the contents of the containers together using the steps outlined below:
   a. Gently swirl the urine in each collection bottle.
   b. Then:

      Option 1: If you have access to a large (e.g., 4 L) flask, beaker, or graduated cylinder, combine the urine from the two collections. Mix gently.

      Option 2: If you do not have access to a large (e.g., 4 L) flask, beaker, or graduated cylinder:

- Gently pour contents from one collection bottle to the other 3 three times, swirling the collections each time to enhance mixing. Even though the entire collection will not be in a single container, the repeated mixing of portions of the collections should allow adequate mixing.

EXAMPLE: a participant brings in 4 L of urine, 3 L in one collection bottle (Bottle A) and 1 L in the other bottle (Bottle B).

a. Measure and record the volumes in each collection bottle.
b. Gently swirl the contents of each bottle to mix the respective samples.
c. Carefully pour 2 L from the Bottle A into Bottle B and gently swirl Bottle B.
d. Carefully pour 2 L from Bottle B into Bottle A and gently swirl Bottle A.
e. Carefully pour 2 L from Bottle A into Bottle B and gently swirl Bottle B.

4. Centrifuge 8 mL of the well mixed urine collection, using the 10 mL tubes provided for urine centrifugation. For QC CCs, centrifuge two additional 8 mL urine samples. Process the 24-hour urine for 5 minutes at 1,300 xg (per *WHI Manuals, Vol. 2 – Procedures; Section 11.6 Urine Processing*).
   - 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. Using a disposable transfer pipette, aliquot the urine by placing:
   - 1.8 mL of urine into each of three labeled 2 mL cryovials.
   - For QC CCs, 1.8 mL of urine into each of six additional labeled 2 mL cryovials (three cryovials for each QC set).

     Be careful not to overfill the cryovials because urine volume will increase upon freezing and the cryovials may crack.
7. Place aliquots in the -70 degree Centigrade freezer.

7.4 Blood Draw Specifics

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

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

7.4.2 Preparation for the Participants

In general, refer to *WHI Manuals: Volume 2 – Procedures; Section 11.2.4.5 – Participant Preparation*.

NBS specifics:

- Each participant must have signed the *NBS Consent* authorizing the CC to draw blood before you can draw her blood.
- Ensure the participant understands the general aspects of blood collection as detailed in the *NBS Consent* and WHI informed consent.
- Verify with the participant that she has been fasting for 6 hours at NBS Visit 2. If needed, refer to *NBS Manual, Section 7.4.4.1 – Directions for Staff When Participants Not Fasted.*

7.4.3 Venipuncture

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

NBS specifics:

- Refer to *NBS Manual, Section 7.2 – NBS Visit 1 (or NBS Visit 3)* and *NBS Manual, Section 7.3 – NBS Visit 2 (or NBS Visit 4)* for the NBS sampling and aliquot schedule.
- Complete the remaining relevant sections of *Form 75 – NBS Visit 1* (for NBS Visit 1), *Form 76 – NBS Visit 2* (for NBS Visit 2), *Form 77 – NBS Visit 3* (for NBS Visit 3), or *Form 78 – NBS Visit 4* (for NBS Visit 4). **Note a missing blood draw in the "Notes" section of the NBS Visit form**. Follow the procedures outlined in the *NBS Manual, Section 8.2.1 – Notes on NBS Forms* to notify the CCC.
- Deliver the blood sample tube(s) and *Form 75 – NBS Visit 1* (for NBS Visit 1) or *Form 76 – NBS Visit 2* (for NBS Visit 2) to the blood processing area. Do the same for NBS Visits 3 and 4 for the reliability subsample, using *Form 77 – NBS Visit 3* or *Form 78 – NBS Visit 4*, respectively.

7.4.4 Blood Collection Problems

7.4.4.1 Directions for Staff When Participants Are Not Fasted

For NBS Visit 2, do not draw the blood sample if the participant has not been fasting for at least 6 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 6 hours before the blood draw.

If she is not fasting and is:

- Willing to wait at the CC until the required number of hours of fasting has occurred, proceed with the blood draw at that time.
- Not willing to wait at the CC 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 then proceed with the remaining NBS Visit 2 tasks. Complete the appropriate section of the *NBS Visit 2 Participant Update Worksheet*.
  - Not willing to reschedule - proceed with the remaining NBS Visit 2 tasks. Complete the appropriate section of the *NBS Visit 2 Participant Update Worksheet* and follow the procedures outlined in the *NBS Manual, Section 8.2.1 – Notes on NBS Forms* to notify the CCC.

7.4.4.2 Handling Participants Who Are Extremely Apprehensive About Having Blood Drawn

Refer to *WHI Manuals: Volume 2 – Procedures; Section 11.2.4.6 - Handling Participants Who Are Extremely Apprehensive About Having Blood Drawn*.

7.4.4.3 Special Consideration for Drawing Blood in the Elderly

Refer to *WHI Manuals: Volume 2 – Procedures; Section 11.2.6.1 - Special Consideration for Drawing Blood in the Elderly.*

7.4.4.4 Difficult-to-Identify Venipuncture Sites

Refer to *WHI Manuals: Volume 2 – Procedures; Section 11.2.6.2 – Difficult-to-Identify Venipuncture Sites.*

7.4.4.5 Difficult Venipuncture

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

NBS specifics:

- The participants may be dehydrated having fasted for 6 hours (except water or black coffee or tea) in preparation for NBS Visit 2. Encourage the participants to drink water liberally during the fast. If this is a particular problem at your CC, consider offering the participants water as they arrive at the visit.
- If venipuncture for NBS Visit 1 (or NBS Visit 3) fails with the second attempt or second technician, do not attempt again. Proceed with the remaining tasks for NBS Visit 1 (or NBS Visit 3). **Note the missing blood draw in the "Notes" section of the NBS Visit form**. Follow the procedures outlined in the *NBS Manual, Section 8.2.1 – Notes on NBS Forms* to notify the CCC.
- If venipuncture for NBS Visit 2 (or NBS 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 *NBS Manual, Section 7.4.4.7 - Deficient Serum and Plasma Samples*). Then proceed with the remaining tasks for NBS Visit 2 (or NBS Visit 4).

7.4.4.6 Fainting

Refer to *WHI Manuals: Volume 2 – Procedures; Section 11.2.6.4 – Fainting.*

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 *NBS Manual, Section 7.5.3.1 Preparation of 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

In general, refer to *WHI Manuals: Volume 2 – Procedures; Section 11.3.1 – Timelimits and Sample Handling.*

NBS specifics:

- The only Vacutainers® being used for NBS are the lavender dry EDTA and royal blue.
- Always protect all the blood samples collected at NBS Visit 2 (or NBS Visit 4) from natural and fluorescent white light. Store samples in covered containers or tubes.

7.5.2 Operating the Refrigerated Centrifuge

Refer to *WHI Manuals: Volume 2 – Procedures; Section 11.3.2 – Operating the Refrigerated Centrifuge.*

7.5.3 Processing Blood Samples

In general, refer to *WHI Manuals: Volume 2 – Procedures; Section 11.3.3 – Processing Samples.*

NBS specifics:

- The only Vacutainers® being used for NBS are the lavender dry EDTA and royal blue.
- Always protect all the blood samples collected at NBS Visit 2 (or NBS Visit 4) from natural and fluorescent white light. Store samples in covered containers or tubes.
- Place the samples in a freezer box dedicated to NBS. Complete the relevant portions of *Form 75 – NBS Visit 1 (*for NBS Visit 1), *Form 76 – NBS Visit 2* (for NBS Visit 2) (and for the reliability subsample; *Form 77 – NBS Visit 3* [for NBS Visit 3], or *Form 78 – NBS Visit 4* [for NBS Visit 4]).

7.5.3.1 Preparation of Blood Cryovials

In general, refer to *WHI Manuals: Volume 2 – Procedures; Section 11.3.3.1 – Preparation of Blood Cryovials.*

NBS specifics:

- 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 75 – NBS Visit 1* (for NBS Visit 1) or *Form 76 – NBS Visit 2* (for NBS Visit Two) to determine the number of cryovials to prepare. Do the same for NBS Visits 3 and 4 with respective NBS Visit forms.

  When transferring the serum and plasma to the cryovials:

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

  - Remove the stopper from the Lavender-stoppered tube.
  - 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 NBS 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 (NBS Visit 2 or NBS 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 NBS 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.
- Complete the relevant portions of the NBS Visit forms.

7.5.4 Freezing and Storing Blood and Urine Cryovials

In general, refer to *WHI Manuals: Volume 2 – Procedures; Section 11.3.5 – Freezing and Storing Blood Cryovials.*

NBS specifics:

- **Store NBS aliquots in boxes dedicated to NBS. Do not mix NBS aliquots with WHI aliquots**.

  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 NBS. Do not store 1.8 mL cryovials in the boxes designated for the 5 mL aliquots.

- Using a black Sharpie® marker, mark NBS in large letters on the side of the top of the box and the side of the bottom of each box.

- Inventory stored aliquots in a way that facilitates shipping all samples from NBS Visit 1 and NBS Visit 2 together for a given participant (and similarly for NBS Visit 3 and NBS Visit 4 for the reliability subsample).

7.5.5 Equipment Quality Assurance

Refer to *WHI Manuals: Volume 2 Procedures; Section 11.3.5.1 – Equipment Quality Assurance.*

7.6 Blood Storage and Shipping

7.6.1 Shipping Schedule

In general, refer to *WHI Manuals: Volume 2 – Procedures; Section 11.4.1 – Shipping Schedule.*

NBS specifics:

- Ship samples from NBS Visit 1 and NBS Visit 2 together for each participant; i.e., delay shipping a participant's Visit 1 samples until her NBS Visit 2 samples have been collected (and similarly for NBS Visit 3 and NBS Visit 4).
  - Within the shipment box, a participant's samples will be in different-sized small boxes (see *NBS Manual, Section 7.5.4 – Freezing and Storing Blood and Urine Cryovials*):
    - The participant's 2mL cryovials (for blood and 24-hour urine) will be in the same type of boxes as used for WHI.
    - The participant's 5mL cryovials (for spot urine) will be in the boxes ordered for NBS (slightly taller than the boxes used for WHI samples).
  - Before sending the shipment, verify that all the specimens (blood, spot urines, and 24-hour urine) have been collected and that all the NBS labels for the participant have the same specimen ID.
- Ship NBS samples in mid- June, August, and November of 2004; and in mid-February and end of March in 2005 (or when all NBS Visits have been completed). Ship to McKesson BioServices on this schedule regardless of the number of samples in the freezer.
- CCs may ship both NBS and WHI boxes together in the same large shipment, but NBS specimens need to be in separate small boxes, marked "NBS".

7.6.2 Mailing Instructions

Refer to *WHI Manuals: Volume 2 – Procedures; Section 11.4.1.1 – Mailing Instructions.*

7.6.3 Packaging Instructions

Refer to *WHI Manuals: Volume 2 – Procedures; Section 11.4.2 – Packaging Instructions*.

7.6.4 Receipt of Frozen Specimen Shipment at McKesson BioServices

Refer to *WHI Manuals: Volume 2 – Procedures; Section 11.4.3 – Receipt of Frozen Specimen Shipment at McKesson BioServices.*

SECTION 8
DATA MANAGEMENT

8.0 Introduction

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

8.1 NBS Label Set

- The CCC provides CCs with NBS 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 NBS Visit 1 and NBS Visit 2.

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

8.2 NBS Data Collection Forms

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

8.2.1 Notes on NBS Forms

- Use the procedures described below to document and handle notes/responses on NBS 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 NBS Forms

- Record notes/responses on NBS Visit forms, worksheets and/or the participant's *Record Sheet for 24-hour Urine Collection* in the following situations:
  - There are missing tasks that prevent data entry of the NBS Visit forms (*Form 75, Form 76, Form 77, or Form 78).*
    - If a specific NBS 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), NBS staff leave the appropriate question on the NBS form 'blank' and indicate the problem in the "Notes" box at the end of the NBS Visit form.
  - There are out-of-sequence tasks that prevent data entry of the NBS Visit form (*Form 75, Form 76, Form 77, or Form 78).*
  - The participant was not given PABA (B-vitamin) tablets because of past hypersensitivity (*Form 75, Form 77).*
  - There are responses on *NBS 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 *NBS Manual, Section 8.2.1.2 – Handling Notes on NBS Forms* for information about handling notes recorded on NBS forms.

8.2.1.2 Handling Notes on NBS Forms

- If staff have a NBS Visit form that cannot be key-entered due to missing or out-of-sequence tasks or if staff or participants record notes as described in *Section 8.2.1.1 - Using the "Notes" Box on the NBS Forms*), then NBS staff should contact the CCC using the steps outlined below:
  1. Make a copy of the entire NBS 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 WHI member ID number).
  3. Contact Helen Penor (hpenor @ whi.org) at the CCC by email or phone to let her know that you are sending a FAX.
  4. Fax a copy of the entire NBS Visit form, *Visit 2 (or Visit 4) Participant Update Worksheet*, or the *Record Sheet for 24-hour Urine Collection* to Helen Penor at the CCC.

8.2.2 Management of NBS Forms

- During NBS Visits, it is critical that all the pages of NBS forms be kept together. The participant member ID is only on the first page of the NBS form, thus it is critical that NBS 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 NBS form) for the 3-hour blood draw.

- All completed and key-entered NBS forms (i.e., *Form 74 – NBS Screening Result, Form 75 – NBS* Visit *1*, etc.) and NBS worksheets (i.e., *NBS Visit 1 Eligibility Checklist Worksheet*, *NBS Visit 2 Participant Update Worksheet*, etc.) are placed in the participant's chart (or special NBS notebook), even when a participant is ineligible or has declined participation.
  - For example: *Form 74 – NBS Screening Result* should be filed in the participant's chart (or special NBS notebook) regardless of the screening outcome (i.e., Scheduled, Ineligible or Declined).

8.3 Data Entry

- The NBS data forms are key-entered in a manner similar to any other WHI form, but NBS forms will have very tight data validation procedures. Staff will not be able to data enter a NBS form if any of the following situations occur: NBS specimen labels do not match, fields on the form are omitted, or a form is entered out of sequence. The reason for these validation procedures is that NBS will happen only once and the timing of the activities is critical. The CCC needs to know that the data is correct, as soon as possible.

8.3.1 Trouble Shooting Data Entry

- This section provides information to help trouble shoot data entry of NBS Visit forms. For information about handling NBS Visit forms that cannot be key-entered due to missing or out-of-sequence tasks, refer to *NBS Manual, Section 8.2.1 – Notes on NBS Forms*.

8.3.1.1 NBS Labels Do Not Match

- If a participant's NBS label on *Form 75 – NBS Visit 1* and *Form 76 – NBS Visit 2* are NOT the same identification number, the form cannot be data entered. If this happens, the CC 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 (NBS Visit 1 and NBS Visit 2).
  - Contact the CCC to help work through the problem.

8.3.1.2 Field Omissions

- WHILMA will not allow a NBS Visit form (*Form 74, 75, 76, 77, 78*) to be data entered if any information is omitted or missing on the form(s). To help prevent potential field omissions and quickly resolve omissions that do occur, CCs are asked to use the thoroughly review the forms and ensure that all required NBS tasks are completed.

  Completeness Checklist and Completeness Staff IDs*:*

  - *Form 75 - NBS Visit* 1 and *Form 77 - NBS Visit 3* have a 'Completeness Checklist' that helps staff review the form. It is important for the NBS-Lead ops (or designee) to review the form and check to see that all NBS visit activities have been completed and the information has been entered correctly on the form. The Lead-ops (or designee) reviewing *Form 75* or *Form 77*, enters their Staff ID on the last page the form, *Qx. 20 – Completeness Staff ID* to indicate that the form has been reviewed and checked for completeness.
  - *Form 76 - NBS Visit 2* and *Form 78 - NBS Visit 4* do not have a completeness checklist, but do have a place to record a 'Visit Completeness Staff ID'. NBS staff who review the form (*Form 76* or *Form 78*) for completeness record their staff ID in *Qx. 15 – Visit Completeness Staff ID*.

8.3.1.3 Time Sequence of NBS Visit Activities

- Spot urines, DLW dosing, meal replacement beverage, and intake of other beverages must be entered in the sequence specified on the NBS Visit form. WHILMA will not allow a NBS Visit form to be data entered if expected tasks are out-of-sequence. For example:
  - A *Form 75 – NBS Visit 1* cannot be data entered if the time(s) entered for *Qx. 18 – Other Beverages Consumed* are earlier than 2 hours post DLW dosing.

8.3.1.4 Forms Entered Out-of-Sequence

- WHILMA will not allow a NBS form to be data-entered out-of-sequence. Checks are in place to enforce the sequence of NBS forms. For example:
  - A *Form 75 – NBS Visit* 1 cannot be data entered unless a *Form 74 – NBS Screening Result* exists indicating that the woman had been scheduled.

8.4 NBS WHILMA Upgrade Notes

- The CCC provides WHILMA Upgrade Notes for the NBS Recruitment Tracking report and data entry of NBS forms. NBS WHILMA Upgrade Notes are located in the *Outlook Public Folders/All Public Folders/Nutri Biomarkers Study*. CCs are encouraged to print a copy of NBS WHILMA Upgrade Notes and place them in the *NBS Manual, Section 8 – Data Management*.

SECTION 9
NBS FORMS AND WORKSHEETS

9.0 Overview

- This section provides copies of the NBS 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 NBS forms and worksheets are included in this section.

9.1 Printing NBS Forms and Worksheets

- CCs will receive 'PDF' files of all NBS Visit forms (i.e., *Forms 74, 75, 76, 77, and 78*) and NBS Worksheets (i.e., *NBS Visit 1 Eligibility Checklist Worksheet, NBS Visit 2 Participant Update Worksheet, NBS Visit 2 (or Visit 4) PABA Worksheet for Staff*). Clinics use these electronic files or the hard copies available in the *NBS Manual, Section 9 – NBS Forms and Worksheets* to make copies of forms and worksheets, as need. For information about NBS printed materials, refer to *NBS Manual, Section 2.4.1 – Supplies Obtained by Each CC*.

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

- *Form 74 – NBS Screening Result*
- *Form 74* Forms Instructions

- *NBS Visit 1 Eligibility Checklist Worksheet*
- *Form 75 – NBS Visit 1*
- *Form 75* Forms Instructions

- *NBS Visit 2 Participant Update Worksheet*
- *NBS Visit 2 (or Visit 4) PABA Worksheet for Staff*
- *Form 76 – NBS Visit 2*
- *Form 76* Forms *Instructions*

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

- *NBS Visit 3 Eligibility Checklist Worksheet*
- *Form 77 – NBS Visit 3*
- *Form 77* Forms Instructions

- *NBS Visit 4 Participant Update Worksheet*
- *Form 78 – NBS Visit 4*
- *Form 78* Forms Instructions

SECTION 10
INDIRECT CALORIMETRY

10.0 Overview of Indirect Calorimetry

- The measurement of resting energy expenditure (indirect calorimetry) will be done in a subset of the clinics participating in the Nutritional Biomarkers Study (Chicago Northwestern, Madison and Pittsburgh). These clinics 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

10.1 Recruitment

- Women participating in NBS at the Chicago-Northwestern, Madison and Pittsburgh clinical centers will be invited. Up to 150 women from all three clinical centers will complete the indirect calorimetry procedures. No additional women will be recruited exclusively for the indirect calorimetry.

- Staff will explain the indirect calorimetry procedures at NBS Visit 1 and those participants who wish to participate will do so at NBS Visit 2 or within a week of their NBS Visit 2 appointment.

- Participants are not obligated to participate in the indirect calorimetry. They may participate in the rest of the Nutritional Biomarkers Study without completing the indirect calorimetry. Participants taking part in the indirect calorimetry will not be offered any additional funds to compensate for time and travel expenses, but they will be offered their resting energy expenditure data (the number of calories their body burns at rest).

10.2 Eligibility

- Women invited to participate in the indirect calorimetry test need to be listed on either the *NBS Recruitment Tracking* report (NBS001) or the *NBS Supplemental Recruitment Tracking* report (NBS002) for expanded NBS recruitment.

- 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.

10.3 Study Procedures

- Clinics may conduct the indirect calorimetry test on the same day as NBS Visit 2 (either pre or post NBS Visit 2) or on a separate day (within a week of NBS Visit 2). For an overview and description of NBS Visit 2 activities, refer to *NBS Manual, Section 5.2 – NBS Visit 2 (Day 15)*.

- The indirect calorimetry procedures will add approximately 1 to 1½ hours to the total time for NBS Visit 2 (total time for both NBS Visit 2 and indirect calorimetry would be about 3 hours).

10.3.1 Pre-Visit 2 Reminder Call

- When the participant's NBS Visit 2 and indirect calorimetry test are on the same day**,** call the participant **two days prior** to the participant's scheduled NBS Visit 2. Confirm the participant's NBS Visit 2 and indirect calorimetry appointments.

- Remind the participant to:
  - **Fast for 12 hours** (instead of 6 hours) prior to her clinic appointment. Ask the participant to stop eating or drinking anything except water (no caffeinated beverages such as black coffee or tea are permitted before indirect calorimetry testing) after 8 PM the night before the scheduled clinic visit for measurement of resting energy expenditure (12 hour fast).

  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."

  - Complete her 24-hour urine collection. Refer to *NBS Manual Section 5.2.2.2.1 – Review Instructions for 24-hour Urine Col*lection for a list of information to cover.
  - Take all her regular medications with water.
  - Wear clothing that 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.

- Ask the participant if she has questions and provide information as needed.

10.3.2 Indirect Calorimetry Procedures

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

- Below is an overview of the steps taken to measure energy expenditure (indirect calorimetry):

  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.
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 <u>but not sleep</u>.
    - 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 3-19) needs to be repeated beginning with a 10 min rest in place of the 30 min called for in the basic protocol.
12. At 30-40 minutes, check the display data printout for a stable reading.
13. End the measurement.
14. Obtain the output data from the metabolic cart.
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 NBS Visit 2 fasting blood draw.**
19. Offer the participant her resting energy expenditure results. Answer any questions.

- **<u>Note:</u>** 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.

10.3.3 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.

10.4 Data Management

- For data collection and management, CCs conducting indirect calorimetry should use the following steps:
  - Obtain a printout of the participant's indirect calorimetry results (minute-by-minute and summary information).
  - Place the participant's "NBS Indirect Calorimetry" label from her NBS label set on the first page of her indirect calorimetry printout.
  - Place the participant's WHI Member ID on each page of her indirect calorimetry printout.
  - Xerox a hard copy of the printout (minute-by-minute and summary information) and staple.
  - Place the xeroxed copy of the indirect calorimetry printout in the participant's NBS chart.
  - Send the original indirect calorimetry printout to Jill Shupe at the WHI Coordinating Center (CCC) by Federal Express at the end of each week (Friday). If you have printouts for more than one participant for that week, send all the printouts in the same Fed-Ex package.
  - Contact Jill Shupe (jshupe@whi.org) each time you send a Fed-Ex package to let her know when the package containing indirect calorimetry results will arrive.

Appendix for Section 10

Indirect Calorimetry

Table of Contents

This appendix provides copies of the materials used in the Indirect Calorimetry subsample of the Nutritional Biomarkers Study.

Contents Page

<WHI Letterhead>
WHI Nutritional Biomarkers Study
Letter of Invitation to Participants-Version 3

<Date>
<Name>
<Address>

Dear <Name>,

Thank you for your participation in the Women's Health Initiative Dietary Study. We are now inviting you to participate in a new research study from the WHI called the WHI Nutritional Biomarkers Study. This study will look at better ways to measure dietary intake of calories, protein, vitamins and minerals. It will also help the WHI scientists better understand the final results from the WHI Dietary Study.

The tests for measuring the nutritional biomarkers are simple. We will ask you to attend two visits at the WHI clinic. You may start the study any time, but if you are due for an Annual Visit, it may be convenient to begin on the same day as your next Annual Visit. The second visit will be two weeks later.

At the first visit, you will be given a special kind of water to drink that will allow us to measure how many calories your body uses during the next two weeks. The special water is tasteless and harmless and contains a type of oxygen and hydrogen that we can measure in your urine.

You will need to come into the clinic after fasting (no food but you may drink liquids) for four hours, then stay at the clinic for five hours and give four urine samples. We will also measure your height and weight, ask you to complete a food frequency questionnaire, questions about any dietary supplements you may use and questions about your usual physical activity. If you are age 60 years or older, we will take a blood sample from a vein in your arm

We will ask you to return to the clinic two weeks later. The day before your return we will ask you to collect all of your urine for 24 hours in a container that we provide for you. You will need to fast for twelve hours before the second visit and it will last three hours.

During the second visit we will take a blood sample, ask you to give two more urine samples, and measure your weight. We will also conduct a breath test that will measure how much energy your body uses at rest. During the breath test we will place a plastic ventilated hood over your head and ask you to lie quietly and breathe normally for 30 minutes.

We will go over these procedures with you again before the study starts.

You should follow your usual eating and activity habits during the two weeks of the study. You will receive a complementary meal at the end of each clinic visit. You will receive $100.00 when you complete the study procedures. You may be invited to repeat the procedures in six months.

You were selected from women who are participating in the WHI Dietary Study. Your name has been used only for the purpose of contacting you and will not be released to anyone. All the information you provide will be kept confidential in accordance with Federal laws.

As usual only your Clinical Center and security-cleared staff at the Clinical Coordinating Center in Seattle, WA will have access to your personal information. Your personal identity will not be revealed in any publications or results. Your participation is voluntary and will not affect your participation in the Women's Health Initiative.

Our clinic staff will telephone you within one week to discuss your interest in participating. We hope you will contribute your time to this important study.

Sincerely,

<CC PI>

CONSENT TO PARTICIPATE IN

THE WOMEN'S HEALTH INITIATIVE (WHI)
NUTRITIONAL BIOMARKERS STUDY

WHI Coordinating Center

Fred Hutchinson Cancer Research Center

Seattle, Washington [Clinical Center]

[Principal Investigator]

[24-Hour Contact]

This consent form is to tell you about the Nutritional Biomarkers Study, which is a new study within the Women's Health Initiative (WHI). You are being asked to take part in this study because you are a participant in the WHI Dietary Study. The purpose of this consent form is to give you information you will need to help you decide whether or not you want to be in the new study. Please read the information carefully. We encourage you to ask questions about the purpose of the research, what we would ask you to do, the possible risks and benefits, your rights as a study participant and anything else about the research or this form that is not clear. When all your questions have been answered, you can decide if you want to be in the study or not. This process is called informed consent.

WHY IS THIS STUDY BEING DONE?

This research is being done because we want to understand about biological markers of foods that women eat. These “biomarkers” are levels of nutrients from foods people eat, such as vitamins, minerals, fat, calories and protein that are found in blood and urine. This research will help the WHI scientists better understand the final results from the WHI Dietary Study.

HOW MANY PEOPLE WILL TAKE PART IN THIS STUDY?

We expect that 550 women who are part of the WHI Dietary Study across the country will participate in this Nutritional Biomarkers Study. Women in the Dietary Change group and women in the Comparison Group are being asked to participate in this new study.

WHAT IS INVOLVED IN THE STUDY?

If you decide to participate, we will ask you to come to your WHI Clinical Center in <Insert name of Clinical Center> for two visits, which will be two weeks apart. While you may begin the study any time at your convenience, the first visit may be the same day as your regularly scheduled WHI Annual Visit, if you are currently due for an Annual Visit. This first visit will last about 5 hours and the second visit, two weeks later will last about 3 hours.

Visit one

Before the first visit we will ask you not to eat food or drink any coffee or tea for four hours before coming to the clinic. You may drink water during these four hours, but coffee or tea may cause dehydration, so we ask that you avoid those beverages during the four hour fast.

During this first clinic visit we will first measure your height and weight and then ask you to drink about ½ cup of a solution called "Doubly Labeled Water." This special water is tasteless, odorless and colorless. It contains naturally occurring special types of hydrogen and oxygen that we can measure in your urine. Drinking the special water on an empty stomach helps it to be absorbed fast. We will ask you to give four urine samples over a four-hour period (one urine sample per hour). These measurements from your urine tell us how much energy (calories) your body uses over the two-week period of the study. If you are age 60 years or older we will take about 2 teaspoons of blood from a vein in your arm at the first visit, about three hours after you drink the doubly labeled water. For all participants, we will ask you to complete a food questionnaire, a dietary supplement use questionnaire, and questions about your usual physical activity. These are questionnaires that you have already completed during other WHI Annual Visits, but they are important to complete again as part of the Nutritional Biomarkers Study. If you are completing your WHI Annual Visit at the same time as the Nutritional Biomarkers Study, you will also complete any of the usual tasks that are part of your WHI Annual Visit during this first visit. For example, this may include a clinical breast exam or a review of medications you may be taking. You may complete the questionnaires and the other Annual Visit tasks in between the urine collections, if necessary.

We will give you a meal replacement beverage (like "Boost" or "Sustacal") one hour after drinking the special doubly labeled water solution and you will receive a complementary meal after you give the fourth urine specimen.

At home between clinic visits

At the end of the first visit we will give you a urine collection kit and explain to you how to collect all of your urine at home for a 24-hour period. You should collect your urine the day before you return for the second visit. You should keep the urine you collect in special bottles we will provide for you and keep them in the refrigerator until you come back to the clinic. You may be asked to take a vitamin pill the day of the 24-hour urine collection. The vitamin should be taken with each meal on the day you collect your urine. This vitamin is called "para amino benzoic acid," or PABA. It is a B-vitamin and is excreted completely in the urine. It will help us know that the all excreted urine has been collected.

Visit Two

Before the second visit we will ask you not to eat anything for twelve hours before coming to the clinic. You may drink water, but no coffee or tea. At the second visit you will bring the urine you collected back to the clinic. Our trained staff will take about two tablespoons of blood from a vein in your arm. Fasting for 12 hours before the blood draw helps us to get

test. We will measure your weight and ask you to give two more urine samples in the clinic, each about one hour apart. The blood samples and urine will be used to measure energy, protein, fats, vitamins and minerals. We will give you a complementary meal or snack when you are done with the tests at the second visit.

During the second visit we will also perform a breath test to measure how much energy your body uses. During the breath test, we will place a ventilated plastic hood over your face and we will ask you to lie quietly for 30 minutes. You will breathe normally during the breath test.

You will receive $100.00 at the end of the second visit for time and travel expenses.

We cannot provide the $100.00 to participants who do not return for the second clinic visit procedures.

If you complete the breath test we will tell you how many calories your body uses each day.

Your participation in this Nutritional Biomarkers Study will be completed at the end of the second visit. However, we are asking 110 women to repeat the study in six months to help us learn how reliable the energy and blood tests are. Participants who repeat the entire study will also complete two 20 minute dietary interviews, one within three weeks after completing the study the first time and the second within about three weeks of the study the second time at the six month point. Trained staff will call you and ask you about all the foods and beverages you consumed the previous day. The dietary interview may sound familiar, as you may have received similar telephone interviews as a participant in the WHI.

We will ask you at the end of the second visit if you want to repeat the study procedures in six months. If you repeat the study you will receive an additional $100.00.

Participation in the Nutritional Biomarkers Study does not affect your participation in the WHI Dietary Study or any other part of WHI.

HOW LONG WILL I BE IN THE STUDY?

You will be in the Nutritional Biomarkers Study for two weeks. You may stop participating at any time.

If you choose to repeat the study in six months, your participation will last again for two weeks.

You will remain a participant in the WHI Dietary Study until it is over in 2005.

WHAT ARE THE RISKS OF THE STUDY?

You may feel dizzy for a few minutes after drinking the special doubly labeled water. To prevent you from feeling dizzy we will ask you to remain seated for about 15-20 minutes after drinking it.

Blood will be taken from a vein in your arm. This 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. If you know you are allergic to PABA, we will not ask you to take it.

You may find it inconvenient to collect urine for a 24-hour period.
If you are claustrophobic or do not like being in small spaces, we will not conduct the breath test if you think you would feel uncomfortable with these procedures.

We do not expect there to be any medical problems as a result of your participation in this study.

If medical problems do occur, we will refer you to your personal physician, or for other appropriate care. You or your health insurance will be responsible for the costs of any such care.

ARE THERE BENEFITS TO TAKING PART IN THIS STUDY?

We hope the information learned from this study will benefit the WHI Dietary Study. The knowledge gained may help scientists learn about blood nutrients and energy needs in postmenopausal women.

WHAT OTHER OPTIONS ARE THERE?

You may choose not to participate in this study.

WHAT ABOUT CONFIDENTIALITY?

All efforts will be made to keep your personal information confidential. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law.

The Office for Human Research Protection and the Institutional Review Board at <insert name of local CC> may have access to participant research records to ensure that participant rights are being met.

We will access your other research records within the Women's Health Initiative, such as Food Questionnaires you have completed in the past and information about your age and race/ethnicity. We will only access personal health information that is part of data that have been collected for the Women's Health Initiative. We will not request any personal health information from other sources, such as hospitals or your physician for the Nutritional Biomarkers Study.

The information from this study may be published in scientific journals or presented at scientific meetings but your identity will be kept strictly confidential.

WHAT ARE THE COSTS?

Taking part in this study may lead to costs to you or your insurance company. Please ask about any expected costs or insurance problems.

In the case of injury or illness resulting from this study, emergency medical treatment is available but will be provided at the usual charges. No funds have been set aside to compensate you in the event of injury.

You or your insurance company will be charged for medical care and/or hospitalization.

WHAT ARE MY RIGHTS AS A PARTICIPANT?

Taking part in this study is voluntary. You may choose not to take part or you may leave the study at any time. Leaving the study will not result in any penalty or loss of benefits to which you are entitled. If you decide to leave the Nutritional Biomarkers Study it will not affect your participation in the WHI Dietary Study.

WHOM DO I CALL IF I HAVE QUESTIONS?

For questions about the study or a research related injury, contact the WHI Clinical Center PI, <name> at <phone number> or any of the investigators listed at the beginning of this form.

For questions about your rights as a research participant, contact the <name of Center> Institutional Review Board (which is a group of people who review the research to protect your rights) at <telephone number>.

INVESTIGATOR'S STATEMENT

My signature below indicates that I have provided an explanation of the above research program. I have explained the risks and benefits of this study and the participant was given an opportunity to ask questions and have them answered. The participant was given an opportunity to discuss the procedures, including possible alternatives and risks, and to ask any additional questions. A signed and dated copy of the consent form has been given to the participant.

_____ _____
Signature of Principal Investigator or Designated Study Staff Date

PARTICIPANT STATEMENT

I have read or had read to me the information provided about this study and have had the opportunity to ask questions about this study. These questions have been answered to my satisfaction. I voluntarily consent to join this new study in the Women's Health Initiative. I understand that any questions I may have about this research in the future will be answered by one of the investigators listed on this form, and that any questions I have about my rights as a research participant will be answered by the persons listed above. I am aware that I and/or my insurance carrier is responsible for any medical costs that may arise from my participation in this research study, including adverse effects. My signature below acknowledges that I voluntarily consent to participate in this study and that have received a signed copy of this consent form.

_____ _____
Signature of Participant Date

_____ _____
Signature of Witness Date

Copies to: Participant

<WHI Letterhead>
WHI Nutritional Biomarkers "Study At A Glance" (Ver. 3)

Thank you for your interest in the WHI Nutritional Biomarkers Study.

Here is what we will ask you to do over the two weeks of the study.

Visit 1 (Day 1)

Come to the WHI Clinical Center having fasted (no food) for four hours. Please bring your dietary supplement bottles.

While fasting, at the visit:

- We will measure your height and weight
- You will give a urine sample
- You will drink the special water solution for measuring the calories you use

One hour later:

You will be given 8 ounces of a liquid meal replacement to drink (Sustacal or similar)

Two through five hours later:

- You will give a urine sample at about 2, 3 and 4 hours after drinking the special water solution – we will tell you when you need to give the urine samples.
- If you are age 60 years or older, we will take a small (2 tsp) blood sample 3 hours after you drink the special water
- You will complete a food questionnaire, a dietary supplement questionnaire, and questions about your usual physical activity

At the end of the first visit:

- You will receive instructions for the second visit.
- You will receive a complementary meal

At home (Day 14)

- Collect all of your urine for the 24-hour period in the special containers we give you. Keep the urine in the refrigerator.
- Take the Special B-Vitamin if asked to do so

Visit 2 (Day 15)

Come to the WHI clinic having fasted (no food) for 12 hours. Bring the urine you collected.

While fasting, at the visit:

- We will measure your weight
- We will take a sample of blood (about 2 Tbsp) from a vein in your arm
- You will give two urine samples – one hour apart – we will tell you when you need to give the urine samples
- You will complete a breath test – we will place a plastic ventilated hood over your head and ask you to lie quietly for about 30 minutes.

Two to three hours later, at the end of the visit:

- You will receive a complementary meal
- You will receive $100.00 for your time and travel
- You will receive information on the number of calories your body uses each day

Please ask the clinic staff if you have any questions

Thank you for your participation in this important study!