

## **Appendix**

**Appendix 1. Overview of biospecimen collection**

**Appendix 2. List of supplies included in the collection kits**

**Appendix 3. Directions provided to participants for blood, urine, time saliva, hair and toenail collection (Collection 1 and 4)**

**Appendix 4. Hair and toenail collection questionnaire**

**Appendix 5. Timed saliva collection log**

**Appendix 6. Blood and urine collection questionnaire**

**Appendix 7: Aliquots created for each fluid sample type in the Mind Body Study**

**Appendix 8. Directions provided to participants for stool and oral microbiome collection (Collection 2 and 3)**

**Appendix 9. Stool and saliva microbiome collection questionnaire**

## **Appendix 1. Overview of biospecimen collection**

Collections 1 and 4 were conducted over three consecutive days; the kits included supplies (**Appendix 2**) and detailed directions (**Appendix 3**). Upon kit arrival, participants were asked to check that all the listed contents were present and to prepare for the collection approximately two weeks later by removing toenail polish, allowing toenails to grow with no polish, and scheduling the blood draw at a local doctor's office or phlebotomy clinic; we referred people to appropriate websites to identify clinics near their location. On Day 1, women were instructed to take a hair sample (if her hair was at least 3 cm in length) and toenail clippings. A small amount of hair (the width of a coffee stirrer) was cut from the center back of the head after securing the hair with a provided elastic band near the scalp. Hair was cut with scissors as close to the scalp as possible without pulling out the roots. For toenails, women were asked to wipe their clippers with a provided alcohol wipe and clip all 10 toes without exposing any sensitive skin. Hair was inserted into a plastic Ziploc bag with a labeled index card and toenails in a labeled coin envelope and stored in a dark place until shipment. A short questionnaire (**Appendix 4**) asked questions about hair and foot care as well as time and date of collection.

On Day 2, women were asked to collect five timed saliva samples into color-coded vials (with straws) over the course of the day; all samples were collected via passive drool. The first collection vial was placed next to the bed the night before to allow for collection immediately upon awaking. Women were asked not to eat, drink, or chew anything for 30 minutes or perform vigorous physical activity for 60 minutes prior to any collection and have no caffeinated beverages before the second collection; we asked that yogurt not be consumed on collection day. Directions (**Appendix 3**) instructed women to collect 2mL saliva samples immediately upon waking, 45 minutes after waking, 4 hours after waking, 10 hours after waking, and at bedtime (before brushing teeth). A saliva collection log (**Appendix 5**) was included to record anticipated and actual collection times and ask about activities and mood at each collection time. Vials were placed into a padded foam pouch in a slide lock plastic bag and kept in the refrigerator until

shipment. At the same time, a cold pack was placed into the freezer and participants called FedEx to set up pick-up for the next day.

On the third day, women began by collecting a first morning urine sample after waking in the morning. After cleaning with a Castile towelette, urine was caught in a large collection cup and then 20mL was aliquoted into a smaller tube with a sealed lid using a disposable pipet. The urine was placed in a biohazard bag in the refrigerator until shipping. Blood collection was conducted at a local clinic using four 10mL sodium heparin tubes and a butterfly needle. Each tube was inverted 10-12 times to prevent blood from clotting before placed into a padded pouch in a biohazard bag. A short questionnaire (**Appendix 6**) asked about date and time of sample collection, medication/hormone use, menopausal status, time since last eating, recent illnesses, weight/recent weight change, and assessment of blood pressure and heart rate. Samples, questionnaires and an ice pack were packed into a box with a biohazard sticker and shipped via overnight courier to our laboratory.

Upon receipt, kits were checked in to our laboratory information system. Before blood samples were processed, a 300uL aliquot of whole blood was taken from one tube and sent the same day to the Boston Children's Hospital Clinical Laboratory for a complete blood count (CBC) with differential to allow for future methylation profiling. The remaining blood was centrifuged for 20 minutes at 2500 RPM and 4 degrees C. Plasma, white blood cells, and red blood cells as well as timed saliva and urine samples were aliquoted into labeled cryovials with screw-top lids and gaskets and stored in liquid nitrogen freezers (**Appendix 7**). Hair and toenail samples were stored in a secure, temperature- and humidity-controlled room in a filing cabinet.

At Collections 2 and 3, participants were asked to provide a stool and saliva sample for microbiome analyses twice 1-3 days apart (**Appendix 8**). We created stool collection devices by assembling a specialized Starstedt tube with a small spoon attached to the lid and 5mL of RNALater (Life Technologies) in a sterilized bio safety cabinet. Saliva was collected using the OMNIGene-Discover kit (DNA Genotek) following the manufacturer's directions. All other

needed supplies (**Appendix 2**) were included in the kit. After the bowel movement, women placed one scoop of stool into each of two collection vials. Samples were placed into a sealed biohazard bag. Women then collected the saliva sample (placed in a slide-lock bag) and completed a questionnaire asking about date and time of sample collection, stool appearance (using the Bristol chart), intake of foods, supplements, and medications that may affect gut flora and oral hygiene (**Appendix 9**). Samples were shipped via FedEx overnight service the day of or after the stool/saliva collection. A second collection using the same procedure, but with a shorter questionnaire along with a 121-item food frequency questionnaire asking about intake over the prior month was completed 1-3 days later. Upon return to the biorepository, kits were checked in and stool samples were processed in a biosafety cabinet, and included homogenization and aliquotting into labeled screw top vials (**Appendix 7**). Saliva samples were similarly aliquotted. All samples were stored at -80°C.

**Appendix 2. List of supplies included in the collection kits.**

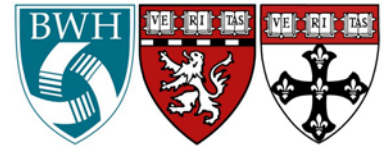
**Contents in each of Collection Kits 1 and 4**

Blood (Quantity)	Urine (Quantity)	Toenail/hair (Quantity)	Timed saliva (Quantity)	Other (Quantity)
<ul style="list-style-type: none"> <li>• 10mL Sodium Heparin (green top) tubes (4)</li> <li>• Surgical gloves (1 pr.)</li> <li>• Butterfly needles (2)</li> <li>• Alcohol wipes (2)</li> <li>• Band-aids (2)</li> <li>• Tourniquet (1)</li> <li>• Gauze pad (1)</li> <li>• Aquipak foam pouch (1)</li> <li>• Leak-proof biohazard bag (1)</li> </ul>	<ul style="list-style-type: none"> <li>• 4-oz. Urine collection cup (1)</li> <li>• Castile Towelette (1)</li> <li>• 20mL Urine holder (1)</li> <li>• Plastic pipette (1)</li> <li>• Slide-lock biohazard bag (1)</li> </ul>	<ul style="list-style-type: none"> <li>• Clear zipper bag with labeled card (1)</li> <li>• Elastic hair band (1)</li> <li>• Alcohol wipe (1)</li> <li>• Envelope for toenails (1)</li> </ul>	<ul style="list-style-type: none"> <li>• 2mL color-capped saliva tubes (5)</li> <li>• Straws (5)</li> <li>• Aquipak foam pouch (1)</li> <li>• Slide-lock bag (1)</li> </ul>	<ul style="list-style-type: none"> <li>• Cover letter (1)</li> <li>• Instructions (1)</li> <li>• Questionnaires (3)</li> <li>• Return shipping kit (1)</li> <li>• Tyvek envelope (1)</li> <li>• Cold pack (1)</li> <li>• Liquid absorbers (2)</li> <li>• Foam pad (1)</li> <li>• Orange biohazard sticker (1)</li> <li>• Return FedEx label (2)</li> </ul>

**Contents in each of Collection kits 2 and 3**

Stool (Quantity)	Untimed saliva (Quantity)	Other (Quantity)
<ul style="list-style-type: none"> <li>• 10mL RNA-later collection tubes with specially designed spoon attached to cap (4)</li> <li>• Latex-free gloves (2 pr.)</li> <li>• Disposable plastic stool collection container with frame (2)</li> <li>• Leak-proof biohazard bag (2)</li> <li>• Trash bag (2)</li> </ul>	<ul style="list-style-type: none"> <li>• OmniGene Discover saliva collection kit (2)</li> <li>• Slide-lock bag (2)</li> </ul>	<ul style="list-style-type: none"> <li>• Cover letter (1)</li> <li>• Instructions (1)</li> <li>• Questionnaires (2)</li> <li>• Food frequency questionnaire (1)</li> <li>• Return shipping kit (2)</li> <li>• Tyvek envelope (2)</li> <li>• Exempt human specimen sticker (2)</li> <li>• Return FedEx label (2)</li> </ul>

# The Nurses' Health Study II



# The Mind Body Study

INSTRUCTIONS FOR THE

HAIR, TOENAIL, TIMED SALIVA, URINE & BLOOD COLLECTION

**Thank you for your generous contribution to this study.**

If you have any questions, feel free to contact us by phone or email.

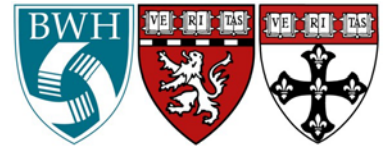
Email us: [NHS2mindbody@partners.org](mailto:NHS2mindbody@partners.org)

Call us: **(617)-525-9204**

For answers to frequently asked questions, visit our website at [\*\*http://www.nhs2.org/mindbody\*\*](http://www.nhs2.org/mindbody)

# The Mind Body Study

HAIR, TOENAIL, TIMED SALIVA, URINE & BLOOD COLLECTION



Thank you again for agreeing to participate in The Mind Body Study!

Please take a moment to look over the contents of this package and make sure you have all materials. This instruction packet contains a Collection Schedule (page 3) and detailed instructions for each segment of the collection (pages 4-12). Packing and shipping instructions are at the end of this packet (pages 13-14).

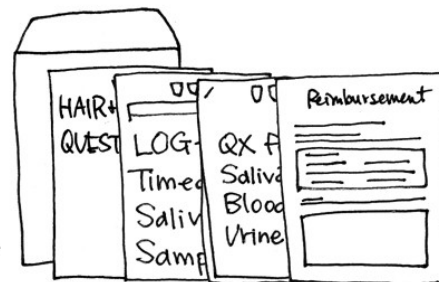
## Kit Contents and Paperwork



DAY 1

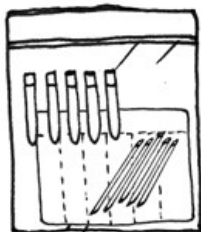


**Hair & Toenail  
Collection Supplies**  
pages 4-6



**Collection  
Paperwork  
Packet**

DAY 2



**Timed Saliva  
Collection Supplies**  
pages 7-8

DAY 3



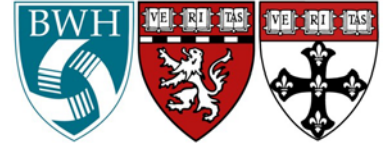
**Urine & Blood  
Collection Supplies**  
pages 9-12



**Packing &  
Shipping  
Supplies**  
pages 13-14

# The Mind Body Study

HAIR, TOENAIL, TIMED SALIVA, URINE & BLOOD COLLECTION



## COLLECTION SCHEDULE

Below is the timeline of collection activities for the Hair, Toenail, Timed Saliva, Urine & Blood Collection.

**Please review this schedule and these requirements carefully before planning your collection.**

### IMPORTANT REQUIREMENTS\*\*\*

- ⇒ **Prepare for your actual collection 2 weeks in advance** by removing toenail polish and growing out your toenails.
- ⇒ **Make an appointment for your blood draw 2 weeks in advance. Blood must be collected Mon, Tues, Wed, or Thurs.**
- ⇒ **Day 1, 2, and 3 must be on consecutive days.** The blood draw will be on Day 3. If necessary, count backwards from the day of your blood draw appointment to see which day you should start your Day 1 activity. *For Day 3 to occur on Monday, Tuesday, Wednesday, or Thursday, Day 1 should occur on Saturday, Sunday, Monday, or Tuesday.*

#### Preparation

*Can be done any day of the week*

- To do list:
- Remove any toenail polish
- Make appointment for blood draw

***(Allow your toenails to grow for approximately 2 weeks)***

#### DAY 1: Hair and Toenail Collection (pages 4-6)

*Must be done Sat, Sun, Mon, or Tues*

- To do list:
- Collect and pack hair and toenail samples
- Complete the Questionnaire for the Hair and Toenail Samples

#### DAY 2: Timed Saliva Collection (pages 7-8)

*Must be done Sun, Mon, Tues, or Wed*

- To do list:
- Collect 5 saliva samples at specified times through the day and refrigerate
- Complete the Log for Timed Saliva Samples
- Prepare for DAY 3 by calling FedEx to schedule a pickup and freezing the cold pack

#### DAY 3: Urine & Blood Collection and Shipping (p 9-12)

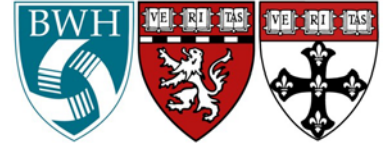
*Must be done Mon, Tues, Wed, or Thu*

- To do list:
- Collect first morning urine and keep cool until you leave for the blood draw
- Take all collection kits, samples, and shipping material with you to the blood draw
- Collect 4 tubes of blood and keep cool until packing
- Complete the Questionnaire for Timed Saliva, Blood, and Urine Samples
- Pack and ship samples via FedEx overnight method (pages 13-14)



# The Mind Body Study

HAIR, TOENAIL, TIMED SALIVA, URINE & BLOOD COLLECTION



## DAY 1: Hair and Toenail Collection

### Preparation

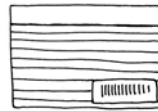
- Remove any nail polish from your toenails and allow them to grow for **TWO WEEKS** before collecting your samples.
- Hair and toenail samples may be collected at any time of the day, but the two samples must be collected on the same day.
- Plan to collect your hair and toenail sample Saturday, Sunday, Monday, or Tuesday (one day before collecting your Timed Saliva and two days before your scheduled blood draw).

### Kit Contents and Paperwork

#### Hair Collection Supplies



Hair elastic



Labeled index card

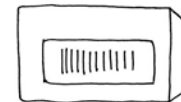


Zip bag for hair sample

#### Toenail Collection Supplies



Alcohol wipe



Labeled coin envelope

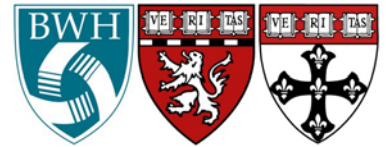
#### Hair & Toenail Paperwork



Questionnaire for the Hair and Toenail Samples  
(Part of Collection Paperwork Packet)

# The Mind Body Study

HAIR, TOENAIL, TIMED SALIVA, URINE & BLOOD COLLECTION



## DAY 1: Hair and Toenail Collection

### Hair Collection Procedure

**NOTE:** It may be helpful to have someone help you with this process!

Your hair must be at least 3cm (approximately 1 1/4" ) long. If your hair is shorter, please skip the hair collection and include a note with your sample shipment.



Separate a strand of hair about the width of a coffee stirrer.

#### 1. DIVIDE HAIR AT THE BACK OF THE HEAD

- Use a hair clip to divide the hair at the back of the head as shown in the figure.

#### 2. SEPARATE OUT ONE BUNDLE OF HAIR

- From the back of the head, separate out one small bundle of hair, about the width of a coffee stirrer.
- Comb the bundle of hair.
- Slip the hair through the provided elastic band.
- Tighten the elastic band around the hair bundle near the scalp.

#### 3. CUT THE HAIR STRAND

- Cut the bundle of hair with scissors as close to the scalp as possible without removing the roots (cut between the elastic band and your scalp).

#### 4. (OPTIONAL) SECURE COLLECTED HAIR WITH TAPE

- If the hair bundle is not securely held by the elastic band, you may wrap a piece of tape around the elastic band and scalp end of the hair sample.

#### 5. PLACE THE HAIR SAMPLE INTO THE ZIP BAG

- Place your hair sample into the zip bag with the labeled index card .

#### 6. STORE THE HAIR SAMPLES IN A DRY AND DARK PLACE UNTIL YOU ARE READY TO PACK AND SHIP.

- Next, collect your toenail samples (page 6) and then store your hair and toenail samples together. These samples will be shipped with your timed saliva, urine, and blood samples on Day 3. Bring them with you when you get your blood drawn.

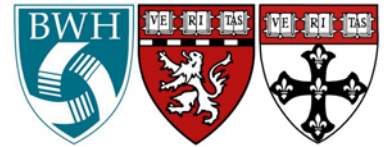
Elastic band



Hair sample in zip bag with labeled index card.

# The Mind Body Study

HAIR, TOENAIL, TIMED SALIVA, URINE & BLOOD COLLECTION



## DAY 1: Hair and Toenail Collection

### Toenail Collection Procedure



#### 1. CLIP ALL 10 TOENAILS

- Wipe your toenail clippers and your toenails with the provided alcohol wipe.
- Clip all 10 toenails with nail clippers. Try to obtain as much toenail as possible without exposing any sensitive skin. If your toenails break while clipping, collect all of the pieces and put them into the provided envelope.



Toenail  
Sample  
Envelope

#### 2. COLLECT THE CLIPPINGS

- Place your clippings in the provided coin envelope and seal the envelope.



Sealed coin  
envelope with  
toenail sample

#### 3. STORE TOENAIL AND HAIR SAMPLES TOGETHER UNTIL DAY 3

- Store the toenail sample with your hair sample in a cool, dark place.
- Be sure to **bring these samples with you when you go to your blood draw**. You will be shipping these samples directly after your blood draw on Day 3. The packing and shipping instructions are on pages 13-14.



Sealed zip bag  
with index card  
& hair sample

#### 4. COMPLETE THE QUESTIONNAIRE FOR THE HAIR AND TOENAIL SAMPLES

- The **Questionnaire for the Hair and Toenail Samples** is located in your **Collection Paperwork Packet envelope**.
- Place the completed **Questionnaire** back into the **Collection Paperwork Packet envelope**.

#### 5. PREPARE FOR DAY 2

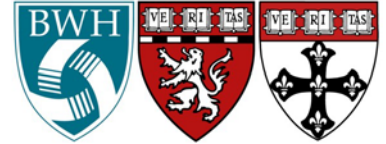
Tomorrow, you will collect five timed saliva samples throughout the day. The first sample will be collected right after you wake up.

- Read over the instructions for Day 2: Timed Saliva Collection (pages 8-9).
- Place the saliva vial for Sample #1 near your bed to make it is easier for you to collect your sample before getting out of bed.



# The Mind Body Study

HAIR, TOENAIL, TIMED SALIVA, URINE & BLOOD COLLECTION



## DAY 2: Timed Saliva Collection

### Preparation

On Day 2, collect **five** timed saliva samples using the schedule below. We will be measuring the amount of cortisol in your saliva and these levels change as part of a normal rhythm during the day. For this reason, it is important to try to collect the saliva samples as close as possible to the collection schedule. Below is a list of important details regarding how best to prepare for the Timed Saliva Collection.

### Notes for the Timed Saliva Collection:

- ⇒ The night before you collect your samples, please place the collection tube for Sample #1 near your bed to make it is easier for you to take your sample before getting out of bed.
- ⇒ Do not eat, drink, or chew anything for at least 30 minutes before each collecting sample.
- ⇒ Avoid all caffeinated beverages until after you have collected Sample #2.
- ⇒ Avoid vigorous physical activity for at least 60 minutes before each collection.
- ⇒ Do not eat yogurt on the day you collect your saliva.

### Timed Saliva Collection Schedule

All 5 of the saliva samples must be collected using the schedule below. Each sample vial has been labeled for a specific time point and color-coded with a cap color that corresponds with the color-coding on the **Log for Timed Saliva**. For each sample you collect, complete the corresponding section on the **Log for Timed Saliva**.

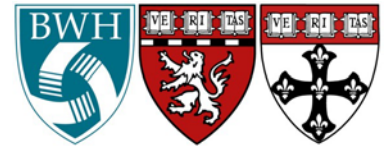
Sample # 1 (GREEN cap)	<b>IMMEDIATELY AFTER WAKING UP</b> (BEFORE getting out of bed or brushing teeth)
Sample # 2 (BLUE cap)	<b>45 MINUTES</b> after waking (BEFORE drinking caffeinated beverages)
Sample # 3 (RED cap)	<b>4 HOURS</b> after waking
Sample # 4 (PINK cap)	<b>10 HOURS</b> after waking
Sample # 5 (YELLOW cap)	<b>JUST PRIOR TO GOING TO BED</b> (BEFORE brushing teeth)

**IMPORTANT:** *For samples #2– #5, please rinse your mouth with cold water before collecting the sample.*

In order to help you plan your day, we suggest that you fill out the *scheduled times* on the Log for Timed Saliva after collecting your first sample. *For example, if you collected Sample #1 at 7:00am, then you should schedule the rest of the day as: Sample #2 : 7:45am; Sample #3: 11:00am; Sample #4 : 5:00pm; and Sample #5: Just prior to going to bed.*

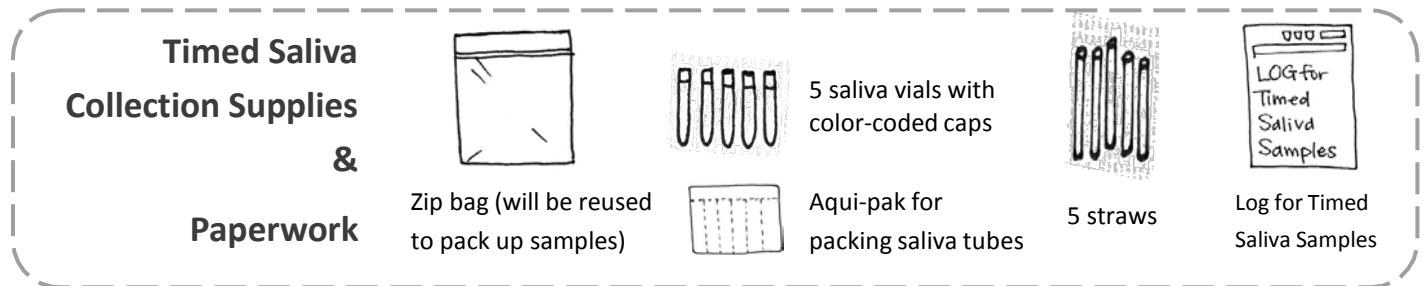
# The Mind Body Study

HAIR, TOENAIL, TIMED SALIVA, URINE & BLOOD COLLECTION



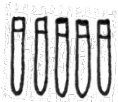
## DAY 2: Timed Saliva Collection

### Kit Contents and Paperwork



### Timed Saliva Collection Procedure

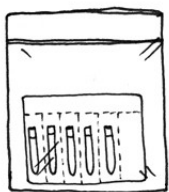
#### 1. COLLECT TIMED SALIVA SAMPLES



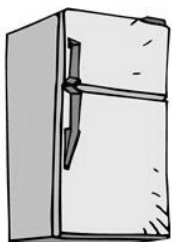
5 color-coded vials



Use straws to help with collection



Refrigerate samples



- Please check that you have the correctly **COLORED** saliva vial for the time point.
- For **Samples #2-5**, rinse your mouth with cold water before collecting the sample
- Collect saliva samples.** Straws are provided to facilitate the collection; it may help to suck in air through the straw and spit saliva through it into the tube.
- Fill the tube to **the 2mL line** with saliva (not just bubbles) . *While filling the vials, if you get too many bubbles, try capping the tube and then tapping the bottom of the tube a few times sharply on a hard surface.*
- Close vial** – Cap the tube securely, ensuring that it is properly threaded.
- Place the vials into the Aquipak absorbent sheet and back into the zip bag.
- Place into the refrigerator as soon as you can** and keep the samples in the refrigerator overnight.

#### 2. COMPLETE THE CORRESPONDING SECTION ON THE LOG FOR TIMED SALIVA.

- For each sample collected, please complete the Log for Timed Saliva. Be sure to include the time the sample was taken (indicating 'AM' or 'PM') and answering the questions.

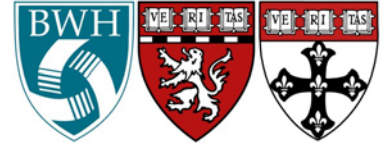
#### 3. PREPARE FOR DAY 3

Tomorrow, you will collect the first morning urine and blood samples, then pack and ship everything — hair, toenail, timed saliva, urine, and blood samples — via overnight method.

- Place the cold pack (found in the white shipping box) in the freezer.
- Call FedEx to arrange for pickup tomorrow (details on page 9).
- Bring all samples, shipping material, and blood collection supplies to the blood draw.

# The Mind Body Study

HAIR, TOENAIL, TIMED SALIVA, URINE & BLOOD COLLECTION



## DAY 3: First Morning Urine and Blood Collec-

### Preparation

#### 2 WEEKS BEFORE the Urine and Blood Collection

**Make appointment for the blood draw**

If you are having your blood drawn at a lab or doctor's office, make an appointment 2 weeks in advance and confirm that they will draw blood for someone in a research study. **The blood draw must occur between Monday and Thursday.** It is fine if the lab uses its own supplies, but ensure that they use **green-top, sodium heparin tubes**. If the lab requires a formal doctor's order, please contact the study staff.

**NOTE:** Some commercial labs, such as Quest and Lab Corp, may have policies that require that they pack and ship your samples for you after the blood collection. In this case, be sure to take all samples and collection and shipping materials with you to the lab (details on page 13). Depending on the lab, you may still need to make your own arrangements to have FedEx pick up samples from the site.

#### ONE DAY BEFORE the Urine and Blood Collection

**Place the cold pack into the freezer overnight**

In preparation for Day 3, place the cold pack in the freezer the night before you plan to collect your samples. The cold pack can be found in the white shipping box.

**Call FedEx**

Call FedEx to arrange for FedEx pickup the samples the day of your urine and blood collection.

Have the provided FedEx airbill (found in the white shipping box) handy when you call. Call 1-800-463-3339 (1-800-GO-FEDEX) and say "schedule a pickup" (or representative" to speak to a live person). You will be shipping via **Priority Overnight** method using a **prepaid stamp** will be asked to provide the pickup address and the send-to address (printed on the airbill).

You may also drop off your parcel at a staffed FedEx location, which can be found by calling FedEx or visiting <http://www.fedex.com/us>. You do not need to make prior arrangements to drop off your samples, but if you plan to go later in the day, it is a good idea to find the cutoff time for dropping off Express shipments at the particular FedEx location.

# The Mind Body Study

HAIR, TOENAIL, TIMED SALIVA, URINE & BLOOD COLLECTION



## DAY 3: First Morning Urine and Blood Collection

### Urine and Blood Collection Timing and Special Restrictions

#### First Morning Urine Sample:

It is most beneficial if your urine sample is a first morning void (if you wake up in the middle of the night to void, collect your sample when you wake up for the day). If you forget to collect a first-morning urine, collect whenever you can.

#### Blood Sample:

Although not essential, we would prefer you to have your blood drawn in the morning before you eat or drink (you can drink water).

### Kit Contents and Paperwork

#### First Morning Urine Collection Supplies



Castile Soap Towelette



Urine Collection Cup



Urine Tube

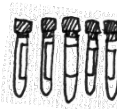


Transfer pipette

#### Blood Collection Supplies



Zip bag (will be reused to pack up samples)



5 green top tubes (1 is extra)



Aqui-pak for packing tubes



2 butterfly needles



Non-latex gloves



Non-latex tourniquet



Gauze pad



Needle holder

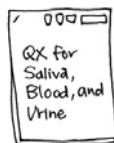


2 alcohol pads



Band-aid

#### Urine & Blood Paperwork



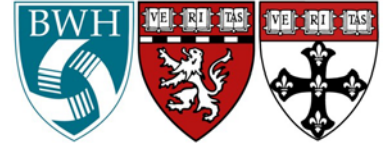
Questionnaire for Timed Saliva, Blood and Urine Samples



Phlebotomy Reimbursement Form

# The Mind Body Study

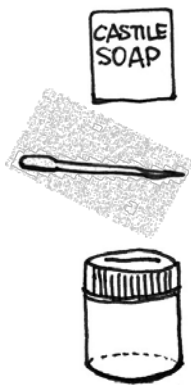
HAIR, TOENAIL, TIMED SALIVA, URINE & BLOOD COLLECTION



## DAY 3: First Morning Urine and Blood Collection

### First Morning Urine Collection Procedure:

It is most beneficial if your urine sample is a first morning void (if you wake up in the middle of the night to void, collect your sample when you wake up for the day). If you forget to collect a first-morning urine, collect whenever you can.



1. **USE THE CASTILE TOWELETTE TO WASH BEFORE COLLECTING THE SAMPLE.**



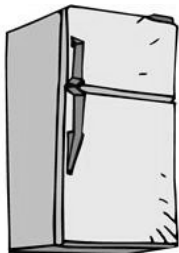
Fill up to  
the line  
marked  
20ML.

3. **TRANSFER URINE INTO TUBE**

- Using the provided pipette, transfer the urine from the collection cup into the smaller urine tube **up to the line marked 20ML**, then recap it.
- Ensure that the cap is threaded properly. Discard the extra urine, the pipette, and the collection cup once you are done filling the urine tube.



4. **STORE URINE**

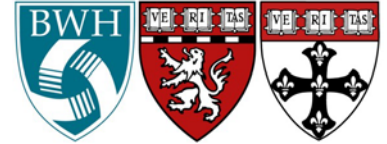


- Place urine sample in Biohazard Bag (found in white shipping box).
- Store urine standing upright (e.g. in a cup) in the refrigerator until you are ready to go have blood drawn.



# The Mind Body Study

HAIR, TOENAIL, TIMED SALIVA, URINE & BLOOD COLLECTION



## DAY 3: First Morning Urine and Blood Collection

### Blood Collection Procedure:

Although not essential, we would prefer you to have your blood drawn in the morning before you eat or drink (you can drink water).



#### 1. BRING ALL COLLECTION MATERIALS TO YOUR BLOOD DRAW

- ⇒ Blood Collection Supplies
- ⇒ Frozen cold pack from the freezer
- ⇒ Shipping supplies in white box
- ⇒ Collection Paperwork Packet (in white Tyvek envelope)
- ⇒ Previously collected samples (hair, toenail, timed saliva, urine)

*Please keep timed saliva and urine samples cool during the day.*

#### 2. COMPLETE the Questionnaire for the Saliva, Blood, and Urine Samples

#### 3. GET BLOOD DRAWN

All supplies needed to draw your blood samples are in your blood collection kit.

- To use the butterfly needle, remove the clear cap. Once the needle is in the vein, push a green-top tube into the clear needle holder until the tube stopper is punctured and blood starts to flow. Once the tube is full, remove it carefully, and gently insert the 2<sup>nd</sup> green-top tube into the holder. Repeat this for the third and fourth tube.
- Immediately after filling each tube, invert it 10-12 times to prevent the blood from clotting.** We have included an extra needle and a fifth tube as an extra in case one fails.
- If you're unable to collect full four tubes, please submit any samples you were able to obtain.
- Instructions for packing up the blood and other samples are on pages 13-14.

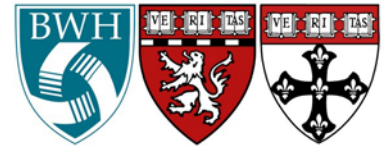
Invert the tube 10-12 times to prevent the blood from clotting.



**If you are charged for the blood draw,** have the laboratory bill the Nurses' Health Study II directly using the reimbursement form included in the kit, or send us a copy of your paid bill for reimbursement using the same form. Please remember to include the receipt. Most charges for blood draw services range from \$5-\$30.

# The Mind Body Study

HAIR, TOENAIL, TIMED SALIVA, URINE & BLOOD COLLECTION



## DAY 3: Packing and Shipping

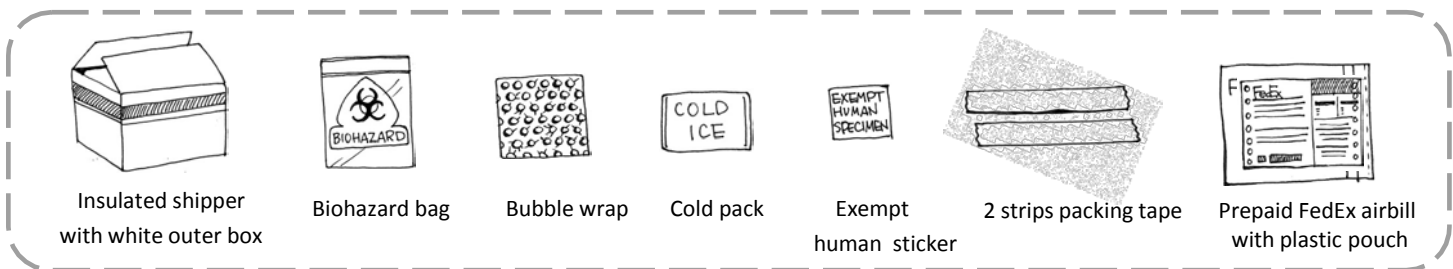
### Preparation

Schedule a FedEx pickup at least one day before you intend to ship your sample. If you are planning to drop the samples off at a staffed FedEx location, find the latest drop-off time for Express shipments. See [page 9](#) for details.

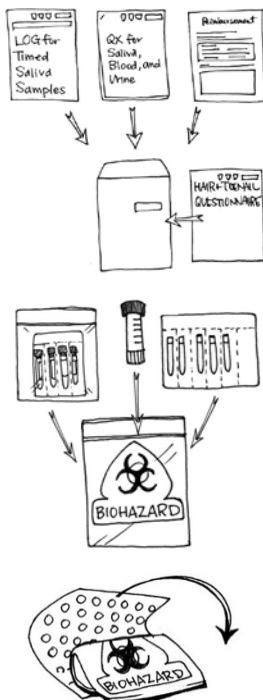
On Day 3, when you go to get your blood drawn, bring with you all the necessary materials for collecting blood and shipping your samples. This includes the blood collection supplies, shipping supplies (outlined below), hair, toenail, timed saliva, and urine samples, as well as the collection paperwork.

**Hint:** If a laboratory will be packing and mailing samples for you, complete step 1 below, then hand the samples and shipping supplies to the laboratory. You may also tear off these pages (13-14) in this packet to give to the laboratory.

### Packing and Shipping Supplies



### Packing and Shipping Procedure



1. Place these items into the **Tyvek Collection Paperwork** envelope and seal:
  - ⇒ **Questionnaire for the Hair and Toenail Collection**
  - ⇒ **Log for Timed Saliva Samples**
  - ⇒ **Questionnaire for the Saliva, Blood, & Urine Collection**
  - ⇒ **Phlebotomy Reimbursement Form** (if requesting reimbursement)
2. **Place the blood tubes** into the 4-pocket AQUI-pak pouch and place back into the clear zip bag.
3. Place the packed blood tubes, the packed timed saliva, and the urine tube into the **Biohazard bag**, and seal.
4. Wrap the Biohazard bag from step 3 with the **sheet of bubble wrap**.

# The Mind Body Study

HAIR, TOENAIL, TIMED SALIVA, URINE & BLOOD COLLECTION



## DAY 3: Packing and Shipping



Toenail sample

Hair sample

Collection papers

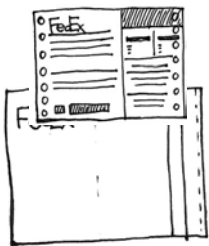
Styrofoam lid

Packed samples

Cold pack

Insulated shipper  
w/white cardboard

EXEMPT  
HUMAN  
SPECIMEN



**FedEx**

5. Place the frozen **cold pack** on the bottom of the **insulated box**.
6. Place the wrapped samples from step 4 (page 13) on top of the cold pack.
7. Close the Styrofoam lid.
8. Place the **envelope containing papers** (from step 1) on top of the lid.
9. Place the **packed hair and toenail samples on top of the lid**.
10. Close the white cardboard box flaps and seal with the **tape**.
11. Affix the **Exempt Human Specimen sticker** to the outside of the box. If it's missing, simply write "Exempt Human Specimen" on the box with a marker.
12. Place the airbill into the **FedEx pouch**, and adhere the pouch to the box.
13. Give the package to the FedEx driver if you have arranged for a pickup, or drop it off at a staffed FedEx location. FedEx has approved this packaging for the shipment of biological samples.

FedEx phone: 1-800-463-3339

FedEx location finder: <http://www.fedex.com/Dropoff/start?cc=us>

*You have completed your Toenail/Hair/Timed Saliva/Urine/Blood Collection.*

***Thank you for your participation!***

# The Nurses' Health Study II

## The Mind Body Study



NHSII Alias ID:  
Kit Number:

### Questionnaire for the Hair and Toenail Samples

Please complete immediately **BEFORE** collecting your hair and toenail samples.

1. **Date of Collection:**    MM/DD/YYYY
2. **Date of Birth:**    MM/DD/YYYY

### Hair Sample Questions

---

3. **Did you wash your hair today before you took your hair sample?**

Yes  No

4. **Before you took your hair sample today, did you use any hair products?**

*Check all that apply*

Hairspray  Hair gel  Mousse  Leave-in conditioner  Other

5. **In the past 3 months, how frequently have you washed your hair per week?**

Less than once  1-4 times  5 or more times

6. **When you wash your hair, do you use:**

Shampoo & Conditioner  Only Shampoo  Only Conditioner

7. **In the past 3 months, have you dyed or bleached your hair?**

Yes   
No



a. The last time you dyed or bleached your hair was it:

At a hair salon

At home

8. **In the past 3 months, have you had any hair treatments other than hair dye?**

*Examples: permanent, straightening, relaxer, etc.*

Yes  No

Toenail Sample Questions



9. In the past 12 months, have you ever polished your toenails?

Yes

No



a. In the past 12 months, how often have you polished your toenails?  
*Check one box for each season.*

	Summer	Fall	Winter	Spring
Weekly	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Every 2 weeks	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Once per month	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Less than once per month	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

b. When you do polish your toenails, how long do you normally keep the polish on?

Less than 1 week     1-4 weeks     More than 1 month

c. Did you need to remove any nail polish from your toes in order to collect your toenail sample?

Yes     No

10. In the past 12 months, have you used nail hardener on your toenails?

Yes     No

11. In the past 12 months, have you applied any medication on your toes?

Yes

No

a. If yes, name of medication:

---

b. Total length of use over the past 12 months:

- Less than 1 week
- 1 – 2 weeks
- 2 – 4 weeks
- 1 – 2 months
- 2 – 3 months
- More than 3 months

## **THANK YOU!**

**Please return this questionnaire with your hair and toenail samples.**

(or mail to: Dr. Walter Willet - Nurses' Health Study II – 181 Longwood Avenue – Boston, MA 02115)

# The Nurses' Health Study II

## The Mind Body Study



NHSII Alias ID:  
Kit Number:

<p><b>Log for Timed Saliva Samples</b></p>	<p>Fill out: </p>	<p><b>Date of Collection:</b> <span style="border: 1px dashed black; display: inline-block; width: 40px; height: 20px; vertical-align: middle;"></span> MM/DD/YYYY</p> <p><b>Date of Birth:</b> <span style="border: 1px dashed black; display: inline-block; width: 40px; height: 20px; vertical-align: middle;"></span> MM/DD/YYYY</p>
--	-------------------	--

Collect your <i>first</i> sample <b>IMMEDIATELY AFTER WAKING UP</b> (BEFORE you brush your teeth)					
1	Time of Collection		Before taking this sample, did you: (within 30 minutes)	Do you feel happy, excited, or content right now?	Do you feel worried, anxious or fearful right now?
		_____ : _____ <input type="radio"/> am <input type="radio"/> pm		<input type="checkbox"/> Brush your teeth? <input type="checkbox"/> Eat anything? <input type="checkbox"/> Drink anything? <input type="checkbox"/> Exercise? <input type="checkbox"/> Get out of bed?	<input type="checkbox"/> Not at all <input type="checkbox"/> Somewhat <input type="checkbox"/> Very much <input type="checkbox"/> Extremely

To help you plan the rest of your day, please fill out the scheduled times below for remaining sample collections.

Collect your <i>second</i> sample <b>45 MINUTES</b> after waking (BEFORE drinking caffeinated beverages)					
2	Time of Collection		Before taking this sample, did you: (within 30 minutes)	Do you feel happy, excited, or content right now?	Do you feel worried, anxious or fearful right now?
		<b>Scheduled</b> _____ : _____ <input type="radio"/> am <input type="radio"/> pm	<b>Actual</b> _____ : _____ <input type="radio"/> am <input type="radio"/> pm	<input type="checkbox"/> Brush your teeth? <input type="checkbox"/> Eat anything? <input type="checkbox"/> Drink anything? <input type="checkbox"/> Exercise?	<input type="checkbox"/> Not at all <input type="checkbox"/> Somewhat <input type="checkbox"/> Very much <input type="checkbox"/> Extremely

Collect your <i>third</i> sample <b>4 HOURS</b> after waking					
3	Time of Collection		Before taking this sample, did you: (within 30 minutes)	Do you feel happy, excited, or content right now?	Do you feel worried, anxious or fearful right now?
		<b>Scheduled</b> _____ : _____ <input type="radio"/> am <input type="radio"/> pm	<b>Actual</b> _____ : _____ <input type="radio"/> am <input type="radio"/> pm	<input type="checkbox"/> Brush your teeth? <input type="checkbox"/> Eat anything? <input type="checkbox"/> Drink anything? <input type="checkbox"/> Exercise?	<input type="checkbox"/> Not at all <input type="checkbox"/> Somewhat <input type="checkbox"/> Very much <input type="checkbox"/> Extremely

Collect your <i>fourth</i> sample <b>10 HOURS</b> after waking					
4	Time of Collection		Before taking this sample, did you: (within 30 minutes)	Do you feel happy, excited, or content right now?	Do you feel worried, anxious or fearful right now?
		<b>Scheduled</b> _____ : _____ <input type="radio"/> am <input type="radio"/> pm	<b>Actual</b> _____ : _____ <input type="radio"/> am <input type="radio"/> pm	<input type="checkbox"/> Brush your teeth? <input type="checkbox"/> Eat anything? <input type="checkbox"/> Drink anything? <input type="checkbox"/> Exercise?	<input type="checkbox"/> Not at all <input type="checkbox"/> Somewhat <input type="checkbox"/> Very much <input type="checkbox"/> Extremely

Collect your <i>fifth</i> sample <b>JUST PRIOR TO GOING TO BED</b> (BEFORE you brush your teeth)					
5	Time of Collection		Before taking this sample, did you: (within 30 minutes)	Do you feel happy, excited, or content right now?	Do you feel worried, anxious or fearful right now?
		<b>Scheduled</b> _____ : _____ <input type="radio"/> am <input type="radio"/> pm	<b>Actual</b> _____ : _____ <input type="radio"/> am <input type="radio"/> pm	<input type="checkbox"/> Brush your teeth? <input type="checkbox"/> Eat anything? <input type="checkbox"/> Drink anything? <input type="checkbox"/> Exercise?	<input type="checkbox"/> Not at all <input type="checkbox"/> Somewhat <input type="checkbox"/> Very much <input type="checkbox"/> Extremely

# The Nurses' Health Study II

## The Mind Body Study



NHSII Alias ID:  
 Kit Number:

### Questionnaire for the Timed Saliva, Blood and Urine Samples

1. Date of Birth    MM/DD/YYYY

Blood Sample		Urine Sample	
2. Date drawn	<input type="text"/> <input type="text"/> <input type="text"/>	4. Date collected	<input type="text"/> <input type="text"/> <input type="text"/>
3. Time drawn	<input type="text"/> <input type="text"/> <input type="radio"/> am <input type="radio"/>	5. Time collected	<input type="text"/> <input type="text"/> <input type="radio"/> am <input type="radio"/>

6. How many hours before the blood drawing did you last eat or drink (not counting water)?  
 Less than 2 hours    2-4 hours    5-7 hours    8-11 hours    12+ hours

7. Was this a first morning urine sample?  
 Yes    No

8. How long were you awake before collecting your urine sample?  
 Less than 1 hour    1-2 hours    3-5 hours    6+ hours

9. In the past 3 months, have you been pregnant or breastfeeding?  
 Yes    No

10. Have your natural menstrual periods ceased PERMANENTLY?

Yes: No menstrual periods

Yes : Had menopause but now have periods induced by hormones

No: Premenopausal

Not sure (e.g. started hormones prior to cessation of periods)

For what reason did your periods cease?

Natural  
 Surgical  
 Radiation or chemotherapy

Age natural periods ceased:

11. Have you had your uterus removed?  
 Yes    No



**12. Have you had either of your ovaries surgically removed?**

No  Yes       How many ovaries do you have remaining?       None       One

**13. Did you smoke cigarettes on the days you collected saliva, blood and urine samples?**

Yes       No


**14. Did you consume beer, wine, and/or liquor on the days you collected saliva, blood and urine samples?**

Yes       No

**15. Did you engage in physical activity long enough to perspire heavily (include swimming) on the days you collected saliva, blood and urine samples?**

Yes       No

**16. Did you drink any caffeinated beverages on the days you collected saliva, blood and urine samples?**

Yes       **Which types?**       Coffee       Tea       Energy drinks  
 No      (Mark all that apply)       Cola       Other carbonated beverage

**17. Have you taken any of the following medications on the days you collected samples?**

- Stimulant medications (Adderall, Ritalin, etc.)  Yes  No
- Beta-blockers (e.g., Propranolol)  Yes  No
- Benzodiazepines  Yes  No
- Oral steroids (e.g. Prednisone)  Yes  No
- Drugs for thyroid disorder  Yes  No
- Antidepressants medication (for any reason)  Yes  No
- Nicotine patch or Nicorette gum  Yes  No
- Antibiotics (don't include topical)  Yes  No
- Aspirin or aspirin products  Yes  No
- Acetaminophen (e.g. Tylenol)  Yes  No
- Ibuprofen (e.g. Advil, Motrin, Nuprin)  Yes  No
- DHEA  Yes  No
- Tamoxifen or Evista (Raloxifene)  Yes  No
- Fosamax or Miacalcin (Calcitonin)  Yes  No
- ACE inhibitors (e.g. Capoten, Vasotec, Zestril)  Yes  No
- Angiotensin receptor blocker (e.g. Diovan, Cozaar, Avapro)  Yes  No
- Other anti-hypertensive (e.g. clonidine, doxazosin)  Yes  No
- Diuretics  Yes  No
- Prilosec, Nexium, Prevacid, Protonix, Aciphex  Yes  No
- H2 blocker (e.g. Pepcid, Tagamet, Zantac, Axid)  Yes  No

**18. Please list the name and dose of any other medications used in the past month:**

**19. In the past two weeks,**

Have you had a cold or other viral or bacterial illness?  Yes  No


Have you had allergies or asthma?

No  Yes, slight  Yes, moderate  Yes, severe

Have you worked night shifts?

No  Yes ( 1-2)  Yes, (3-4)  Yes (5-6)  Yes, (7+ shifts)

**20. In the past 6 months, have you used female replacement hormones (other than oral contraceptives)?**

Yes  **1. Are you currently using them (within the past month)?**  
 Yes, currently  No, not currently go on to # 8

No  


**2. What hormones have you used in the past month?**  
(If you are taking multiple hormones, fill in all that apply)

Estrogen  Oral  
Brand: \_\_\_\_\_  Patch  
 Other: \_\_\_\_\_


Progesterone  Oral  
Brand: \_\_\_\_\_  Patch  
 Other: \_\_\_\_\_

Testosterone  Oral  
Brand: \_\_\_\_\_  Patch  
 Other: \_\_\_\_\_

Any other hormone preparation:  
Brand: \_\_\_\_\_

Oral  Patch  Other: \_\_\_\_\_

**21. In the past 3 months, have you used a soy or herbal estrogen product 4+ days per week?**

Yes  **Which types?**  Soy milk/bars  Soy pills/ powder  
 No (Mark all that apply)  Black cohosh  Tofu  Other: \_\_\_\_\_

**22. Current Weight (lbs.):**

**23. Has your weight changed during the past 3 months?**

Appendix 6 – Blood, Urine and Timed Saliva Questionnaire

No change (less than 5 pounds gained or lost)

Lost 5-10 pounds

Lost 10 + pounds

Gained 5-10 pounds

Gained 10 + pounds

**24. If you own your blood pressure cuff and were able to record your blood pressure right before you went to sleep, please enter you recorded blood pressure information below.**

BP measurement:	<input type="text"/>	systolic BP/diastolic BP
Heart rate:	<input type="text"/>	bpm
Time Taken:	<input type="text"/>	<input type="radio"/> am
		<input type="radio"/> nm

**Thank you!**

**Please return this questionnaire with your blood, urine and saliva samples.**

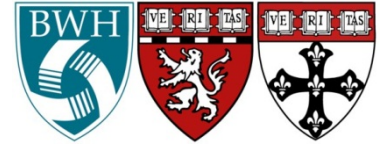
(or mail to: Dr. Walter Willet - Nurses' Health Study II – 181 Longwood Avenue – Boston, MA 02115)

**Appendix 7: Aliquots created for each fluid sample type in the Mind Body Study**

<b>Sample type</b>	<b>N, 4.5mL aliquot</b>	<b>N, 1.8mL aliquot</b>	<b>N, lab-ready vial*</b>
Blood, Plasma	2	3	3
Blood, white blood cell	NA	2	1
Blood, red blood cell	1	NA	NA
Urine	4	NA	2
Timed saliva (each timepoint)	NA	1	1
Stool	NA	1	1
Untimed saliva	NA	1	1

\*Lab-ready vials ranged in size from 100ul (plasma) to 1.8mL (stool & untimed saliva)

# The Nurses' Health Study II



# The Mind Body Study

## INSTRUCTIONS FOR THE STOOL AND SALIVA COLLECTION

**Thank you for your generous contribution to this study.**

If you have any questions, feel free to contact us by phone or email.

Email us: [NHS2mindbody@partners.org](mailto:NHS2mindbody@partners.org)

Call us: (617)-525-9204

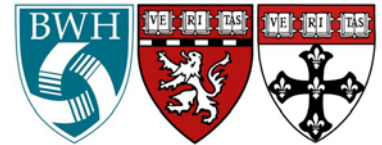
For answers to frequently asked questions, visit our website at <http://www.nhs2.org/mindbody>

Email: [nhs2mindbody@partners.org](mailto:nhs2mindbody@partners.org) | Phone: (617)-525-9204 | Web: <http://www.nhs2.org/mindbody>

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# The Mind Body Study

## STOOL AND SALIVA COLLECTION



Thank you again for your participation in **The Mind Body Study!**

For this portion of the NHSII Mind Body Study, we are requesting two sets of stool and saliva samples collected 1-3 days apart, along with associated questionnaires.

Set #1 collection and shipping materials are labeled with NEON labels. Set #2 materials are labeled with WHITE materials. Please ensure that you use the correct materials for each collection.

## COLLECTION SCHEDULE

Please collect samples any day of the week EXCEPT Saturday. Collect Sample Set # 1 within 4 weeks of receiving the kit. Complete Sample Set #2 within 1-3 days after Set #1. **To ensure sample integrity, please plan to ship the samples one day after collection.** If you have recently been taking antibiotics, please postpone collection for 2 –3 weeks to allow your fauna to re-establish itself before collecting your samples.

### Collect Sample Set # 1 *Within 4 weeks of receiving the kit; any day EXCEPT Saturday*

*All components of Sample Set # 1 are labeled with NEON labels.*

- To do list:
- Collect 2 vials of stool using the vials with **NEON** labels (p.6-7)
- Collect 1 vial of saliva using the OMNIgene collection kit with a **NEON** label (p.7-8)
- Complete the Lifestyle Questionnaire and Collection Data Sheet Set #1
- Pack samples and paperwork and store at room temperature (p.10)
- Call FedEx and arrange for them to come pick up the samples the following day (p.10)

***Wait 1 –3 days before collecting the next set of samples.***

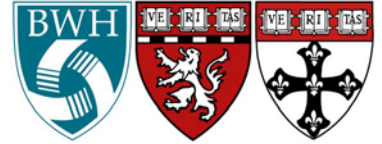
### Collect Sample Set # 2 *1– 3 days after the first collection; any day EXCEPT Saturday*

*All components of Sample Set # 2 are labeled with WHITE labels.*

- To do list:
- Collect 2 vials of stool using the vials with **WHITE** labels (p.6-7)
- Collect 1 vial of saliva using the OMNIgene collection kit with a **WHITE** label (p.7-8)
- Complete the Food Frequency Questionnaire
- Complete the Collection Data Sheet # 2
- Pack samples and paperwork and store at room temperature (p.11)
- Call FedEx and arrange for them to come pick up the samples the following day (p.11)

# The Mind Body Study


## STOOL AND SALIVA COLLECTION



### Kit Contents: Stool and Saliva Sample Set # 1 (NEON labels)

Please plan to collect Sample Set # 1 within 4 weeks of receiving the kit. Do not collect samples on Saturday.

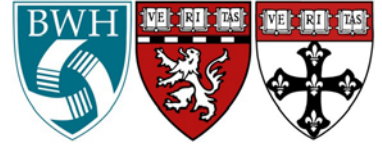
NOTE: All Set #1 collection and mailing materials and paperwork are included in the Set #1 Mailing Box. All components of Sample Set # 1 are labeled with a NEON label.

Mailing Box, Set #1 (9x4x4")	2 stool vials, Set #1 <b>(NEON labels)</b>	Biohazard bag for packing stool vials	Saliva collection kit, Set #1 <b>(NEON label)</b>	Zip bag for packing saliva vial
Latex gloves	Tyvek Envelope for packing Questionnaire <b>(NEON label)</b>	Lifestyle Questionnaire and Collection Data Sheet, Set #1 <b>(NEON label)</b>	FedEx Shipping Label and Pouch	Packing tape
<b>Paperwork: Sample Set #1 (NEON labels)</b>			 <p data-bbox="1003 1619 1333 1687">Commode for stool collection (included in large mailing box)</p>	
Exempt Human Specimen Sticker	Trash bag for disposing of commode			



# The Mind Body Study

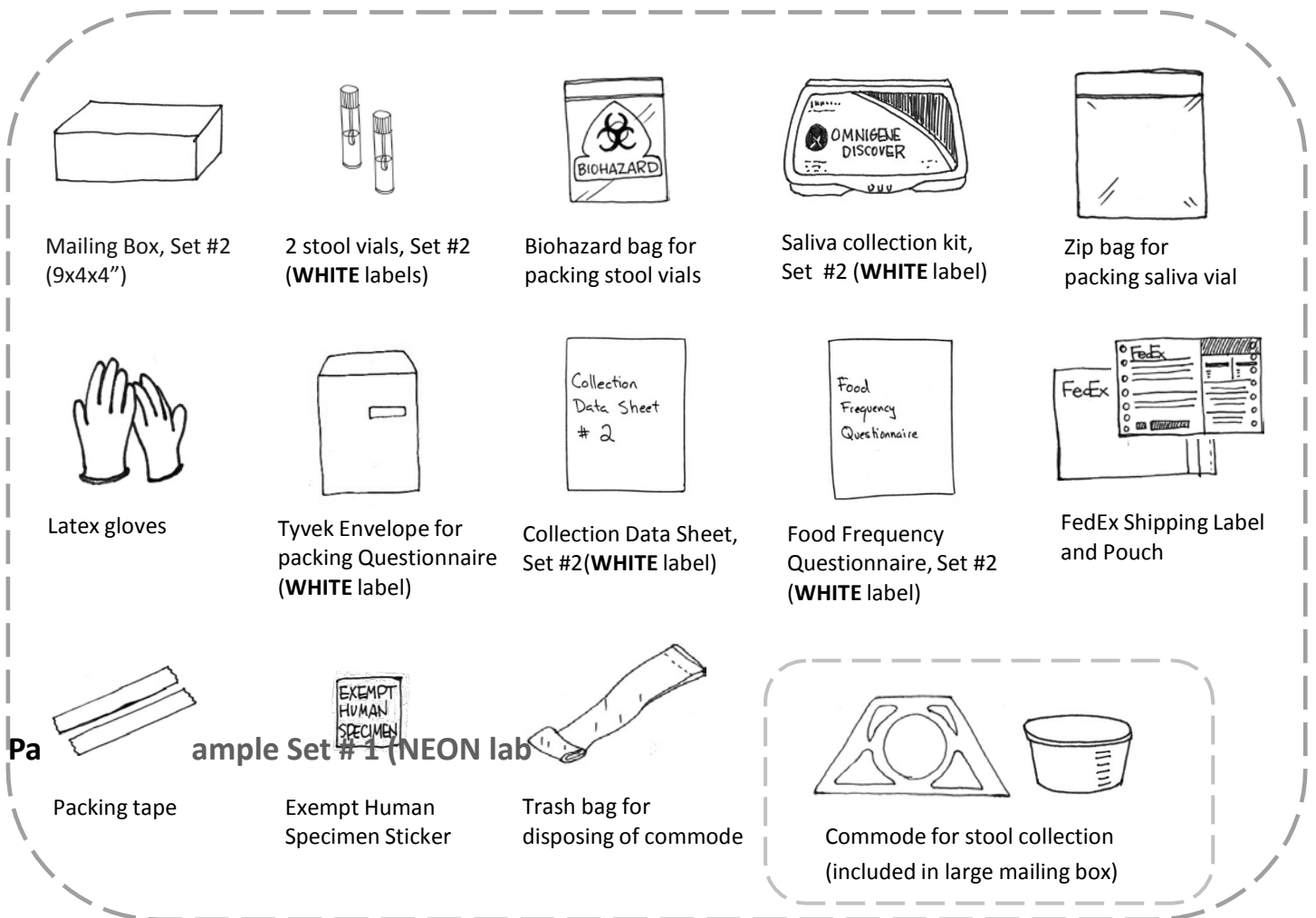
## STOOL AND SALIVA COLLECTION



### Kit Contents: Stool and Saliva Sample Set # 2 (WHITE labels)

Please plan to collect Sample Set # 2 within 1– 3 days after Sample Set #1 is collected. Do not collect samples on Saturday.

NOTE: All Set #2 collection and mailing materials and paperwork are included in the Set #2 Mailing Box. All components of Sample Set # 2 are labeled with a WHITE label.



# The Mind Body Study

## STOOL AND SALIVA COLLECTION



### Stool Sample Collection Procedure:

The following describes the procedure for collecting two vials of stool. Follow the same procedure for collecting samples for Set #1 (NEON labels) and Set #2 (WHITE labels), ensuring that the correct color-coded supplies are used for the particular Sample Set.



#### 1. SET UP THE COLLECTION COMMODORE

- Raise the toilet seat.
- Place the stool collection frame on the back of the toilet bowl (see **Figure 1**). All four corners of the collection frame should be supported by the toilet bowl.
- Place toilet seat down (see **Figure 2**).
- Place collection bowl in frame (see **Figure 2**).



#### 2. COLLECT THE STOOL

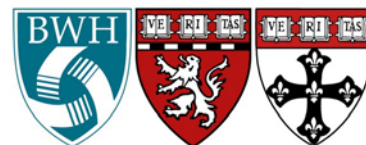
- The collection unit should look like **Figure 3** when assembled.
- Do not urinate** into the collection commode.
- Deposit your stool directly into the collection commode.

**NOTE:** If you accidentally urinate into the commode, discard the stool and collect a new sample using the second commode provided. Please call us for additional supplies to be sent Express to your home.



# The Mind Body Study

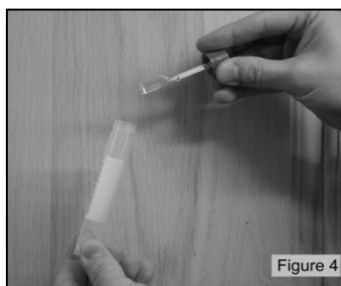
## STOOL AND SALIVA COLLECTION



### Stool Sample Collection Procedure (continued):

#### 3. TRANSFER ONE SPOONFUL OF THE STOOL INTO EACH STOOL VIAL

Collect 2 vials of stool for each Sample Set. The stool vials have built-in spoons to help you obtain sample.



Again, be sure to use correct color coded vials.

Sample Set # 1: **NEON** labels that read "Stool vial, Set #1"

Sample Set # 2: **WHITE** labels that read "Stool vial, Set #2"

- Unscrew the stool vial cap with built-in spoon (**Figure 4**).
- Collect **ONE** spoonful of the stool starting at the surface and pushing into the center of the stool sample in the commode. *It is fine if the fixative drips on the stool.*
- Place the spoonful of collected stool into the vial. **DO NOT** fill the vial with additional amounts of stool.
- Screw the cap on tightly to close the vial.
- Repeat this procedure for the second vial.
- Place these vials in the Biohazard zip bag and seal the bag.
- Store the samples at room temperature.
- Follow the instructions for packing up the samples.

- Sample Set #1 packing instructions : [page 10](#)

- Sample Set #2 packing instructions: [page 11](#)

**NOTE:** These vials contain a fixative that can cause irritation if it comes in contact with the skin. If contact occurs, rinse with water.

#### 4. DISCARD THE COLLECTION CONTAINER

- Leaving the remaining specimen in the commode, remove the commode from the frame (see **Figure 5**).
- Close the lid of the commode.
- Discard the commode and the frame with your normal trash. Trash bags are provided for this purpose.

#### 5. NEXT STEPS

- Collect your saliva specimen on the same day. (pages [8-9](#))
- Call FedEx to arrange pickup the following day.



# The Mind Body Study

## STOOL AND SALIVA COLLECTION



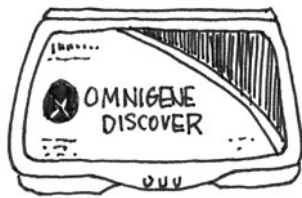
### Saliva Sample Collection Procedure:

Collect **ONE** tube of saliva specimen on the same day as stool.

Your saliva specimens can be collected **before or after** the stool.

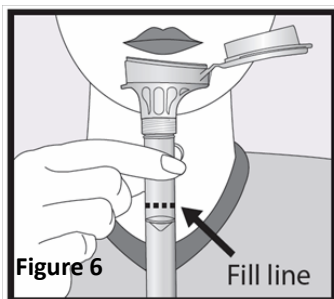
These saliva samples can be collected at any time of the day. However, **DO NOT EAT, DRINK, SMOKE, CHEW GUM, OR CLEAN YOUR TEETH for at least two hours before collecting your saliva** (you may drink water). If you collect your saliva first thing in the morning, please do so prior to eating breakfast and brushing your teeth.

**NOTE:** The Omnigene vial contains a fixative that can cause irritation if it comes in contact with the skin. If contact occurs, rinse with water.



#### 1. PREPARATION

- The OMNigene saliva collection kit should be kept at room temperature.
- Be sure to select the correct color coded vial for the Sample Set:
  - Sample Set # 1: **NEON** label that reads “Saliva vial, Set #1”
  - Sample Set # 2: **WHITE** label that reads “Saliva vial, Set #2”



Spit until the amount of saliva (not bubbles) reaches the fill line.

#### 2. COLLECT THE SALIVA SAMPLE

- Remove the collection vial from the clamshell package. **DO NOT TRY TO CLOSE THE VIAL CAP AT THIS TIME. DO NOT REMOVE THE PLASTIC FILM FROM THE INSIDE OF THE CAP.** The preservative is located in the cap and should be released only after all the saliva sample has been collected in the vial.
- Collect your saliva sample by spitting through the funnel attached to the vial (see Figure 6).
- Spit until the amount of liquid saliva (not the bubbles) reaches the **fill line** as shown in the figure. If the vial is full of bubbles, tap the vial against a table to break them up.

**Hint:** To produce more saliva, close your mouth and wiggle your tongue (without rubbing your tongue on your cheeks) or rub your cheeks with your hands. Complete spitting/collecting within 30 minutes from when you began.

# The Mind Body Study

## STOOL AND SALIVA COLLECTION



### Saliva Sample Collection Procedure (continued):

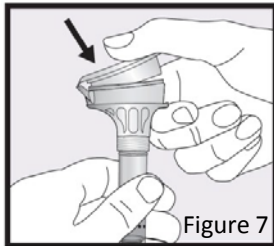


Figure 7

Close lid tightly by pushing down hard on the funnel lid until you hear a loud click.

### 3. CLOSE THE SALIVA VIAL

- Once you have filled the saliva vial to the fill line, keep the vial upright and place it on a flat surface.
- Close the funnel lid tightly by pushing down hard on the lid until you hear a click, as shown in **Figure 7**.
- Be sure that the cap is closed tightly as shown in **Figure 7**.

**Hint:** If you have trouble closing the cap all the way, readjust the angle of the funnel lid and push again on the lid to fit it onto the vial. You should see the liquid from the lid being released into the vial.

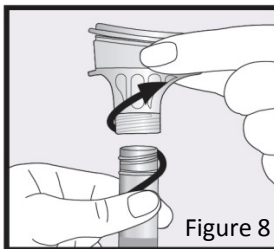


Figure 8

Unscrew the funnel from the tube.

- Unscrew the funnel from the vial, as shown in **Figure 8**.
- Close the vial with the round cap provided in the clamshell kit, as shown in **Figure 9**. Be sure that the cap is threaded and closed tightly.
- Mix gently for at least 10 seconds, holding the vial as shown in **Figure 10**.
- Store the closed saliva vial at room temperature: 59-86°F (15-30°C).
- Place this vial in the absorbent Aquipak sleeve, then into the plain zip bag.
- Follow the instructions for packing up the samples.

- Sample Set #1 packing instructions : [page 10](#)

- Sample Set #2 packing instructions: [page 11](#)

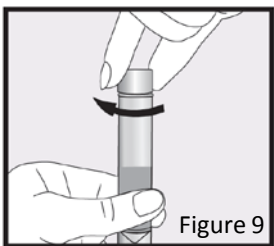


Figure 9

Close tube tightly with small cap.

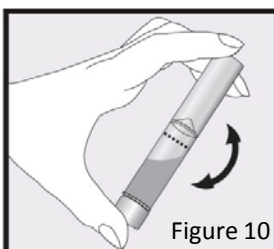


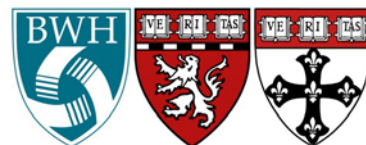
Figure 10

Shake for 10 seconds.

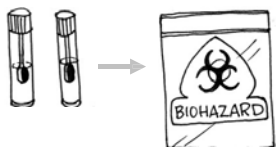
The funnel and the OMNIgene collection kit packaging can be discarded in your regular trash.

# The Mind Body Study

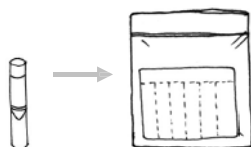
## STOOL AND SALIVA COLLECTION



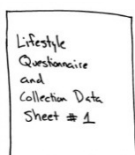
### Packing and Shipping Procedure for Sample Set #1 (NEON labels):



1. Place the 2 stool vials in the Biohazard bag, seal, and place in the 9x4x4 mailing box.

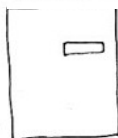


2. Place the 1 saliva tube in the plain zip bag with the Aquipak, and seal, and place in the 9x4x4 mailing box.

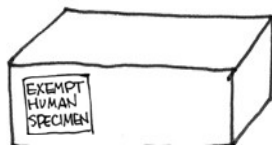


3. Complete the "Lifestyle Questionnaire and Collection Data Sheet, Set #1."

4. Place the completed form from step 3 in the white Tyvek envelope and seal. Place the envelope in the 9x4x4 mailing box.



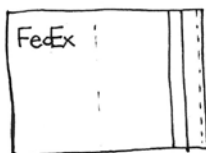
5. Place the Exempt Human Specimen sticker on the outside surface of the shipping box (if it is missing, you may write "Exempt Human Specimen" on the box with a marker).



6. Seal the shipping box with the provided tape.



7. Complete the shipper's information (section 1) on the FedEx airbill. Insert the airbill into the provided FedEx pouch, and adhere the pouch onto the box.

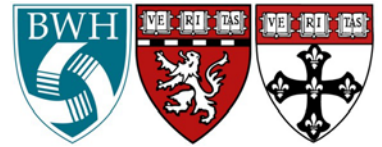


8. Call FedEx to schedule a pickup for the following day. Dial 1-800-463-3339 (1-800-GO-FEDEX) and say "schedule a pickup." You will be shipping via Standard Overnight method and will be asked to provide the pickup address and the ship-to address (printed on the provided airbill). You may also drop off your samples at a staffed FedEx location.



*To ensure sample integrity, please ship the samples one day after collection, unless the collection falls on a Saturday. If you collect on a Saturday, ship on Monday.*

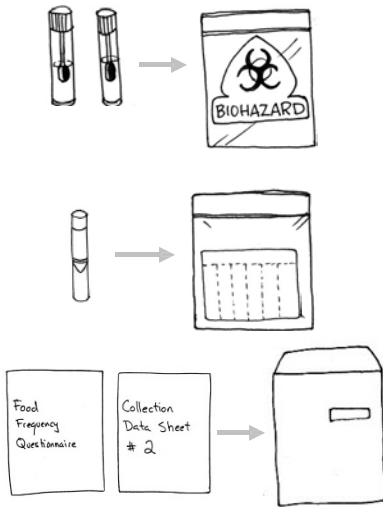
*These samples may be shipped Friday and Saturday; however, please note that FedEx does not pick up on Saturdays (drop off at staffed locations only).*



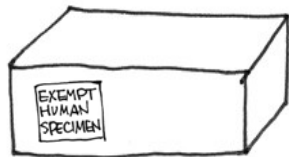
# The Mind Body Study

## STOOL AND SALIVA COLLECTION

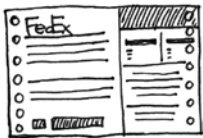
### Packing and Shipping Procedure for Sample Set #2 (WHITE labels):



1. Place the 2 stool vials in the Biohazard bag, seal, and place in the 9x4x4 mailing box.
2. Place the 1 saliva tube in the plain zip bag with the Aquipak, and seal, and place in the 9x4x4 mailing box.
3. Complete the "Food Frequency Questionnaire" and the "Collection Data Sheet, Set #2."
4. Place the completed forms from step 3 in the white Tyvek envelope and seal. Place the envelope in the 9x4x4 mailing box.



5. Place the Exempt Human Specimen sticker on the outside surface of the shipping box (if it is missing, you may write "Exempt Human Specimen" on the box with a marker).
6. Seal the shipping box with the provided tape.
7. Complete the shipper's information (section 1) on the FedEx airbill. Insert the airbill into the provided FedEx pouch, and adhere the pouch onto the box.



8. Call FedEx to schedule a pickup for the following day. Dial 1-800-463-3339 (1-800-GO-FEDEX) and say "schedule a pickup." You will be shipping via Standard Overnight method and will be asked to provide the pickup address and the ship-to address (printed on the provided airbill). You may also drop off your samples at a staffed FedEx location.

*Again, to ensure sample integrity, please ship the samples one day after collection.*

*You have completed the Stool and Saliva Collection.*

***Thank you again for your participation!***

**The Nurses' Health Study II**  
*The Mind Body Study*



NHSII Alias ID:  
 Kit Number:

**Lifestyle Questionnaire and Collection Data Sheet, #1**  
**Sample Set # 1**

**PLEASE COMPLETE AFTER COLLECTING YOUR SAMPLE SET**

The following information will be used to do a comparative analysis between your food intake, your digestive transit time, and the microbiota (bacterial composition) of your digestive tract.

1) **Date of Birth**    MM/DD/YYYY

Saliva Sample, Set # 1	Stool Sample, Set # 1
------------------------	-----------------------

2) **Date drawn**

4) **Date collected**

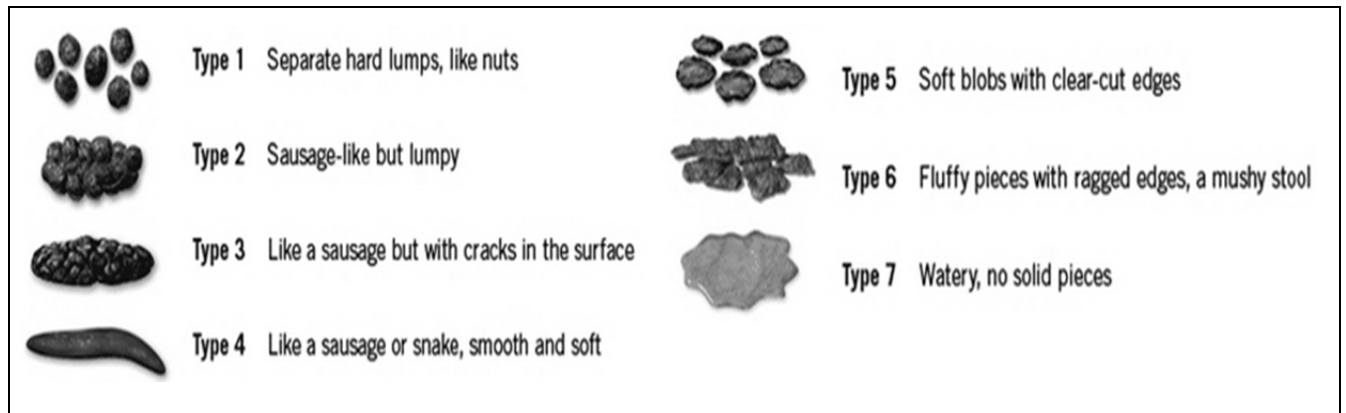
3) **Time drawn**    am  
 pm

5) **Time collected**    am  
 pm

6) **Was the collection done prior to breakfast and washing teeth?**  
 Yes  No

7) **Based on the attached Bristol chart, what was the appearance of the stool at the site you collected the sample to put into the tube (SET 1)?**  
 Type 1     Type 2     Type 3     Type 4     Type 5     Type 6     Type 7  
 Something between Type \_\_\_ and Type \_\_\_

**Bristol Chart**



8) **In the past 12 months, please check if have you received any of the following medications as pills or through the vein: (DO NOT INCLUDE INHALERS OR TOPICAL CREAMS)**  
 Antibiotics     Chemotherapy     Immunosuppressant's (e.g. oral corticosteroids)

9) **In the past 2 months, have you undergone a colonoscopy or other procedure requiring bowel preparation?**  
 Yes  No

10) **In the past 2 months, have you used an oral contrast agent for a CT scan or x-ray?**  Yes  No



11) In the past 2 months, have you had diarrhea?  Yes  No

12) In the past 2 months, have you been hospitalized for any reason?  Yes  No

13) In the past 2 months, have you had any acute diarrheal illnesses?  Yes  No

14) In the past 2 months, have you consumed any probiotics (other than yogurt; see below) at least once per week?  
 Yes  No

15) In the past 2 months, how often have you consumed yogurt or other foods containing active bacterial cultures (kefir, sauerkraut, etc.)?  
 Never  Rarely  1-5 times a week  Daily  More than daily

16) What are your dietary preferences with respect to meat?

- Standard diet  Vegetarian (no meat)  
 Standard diet with poultry and/or fish (no red meat)  Vegan (no meat, dairy, or animal products)

17) How often do you consume alcoholic beverages?

- Never  Rarely  1-5 times a week  Daily  More than daily

18) In the past 2 months, have you used any acid reducing medications chronically (> 1 week), including (but not limited to): Omeprazole (e.g. Prilosec, Zegerid), Protonix (e.g. Pantoprazole), Esomeprazole (e.g. Nexium, Vivomo), Lansoprazole (e.g. Prevacid), Dexlansoprazole (e.g. Dexilant), Ranitidine (e.g. Zantac), Famotidine (e.g. Pepcid), Nizatidine (e.g. Axid), Cimetidine (e.g. Tagamet)?

- Yes  No

19) In the past 2 months, have you used any medications modifying bile production chronically (> 1 week), including (but not limited to): Cholestyramine (e.g. Questran, Prevalite, Locholest), colestipol (e.g. colestid), colesevelam (e.g. Welchol), chenodeoxycholic acids (e.g. CDCA), or ursodeoxycholic acid (e.g. UDCA, Ursodiol, Actigall)?

- Yes  No

20) How often do you use the following oral hygiene products:

	Never	Rarely	1-5/week	Daily	More than 1/day
Manual toothbrush	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Electric toothbrush	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Alcohol-based mouthwash	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Non-alcoholic mouthwash	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Floss	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Water-based pick/jet	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Tongue cleaner	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Tooth-whiteners	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

21) Have you seen a dentist (generalist, prosthodontist, periodontist, etc.) within the last:

- Month  6 months  Year  2 years  More than 2 years  NA

22) Have you had a professional dental cleaning (dentist, hygienist, etc.) within the last:

Month  6 months  Year  2 years  More than 2 years  NA

**23) If you wear dentures, how often do you clean them?**

Don't own  Rarely  1-5 times a week  Daily  More than once daily

**24) Do you think you might have gum disease?**

Gum disease (or periodontal disease) is a common problem with the mouth. People with gum disease might have swollen gums, receding gums, sore or infected gums, or loose teeth.

Yes  No  Don't know

**25) Overall, how would you rate the health of your teeth and gums?**

Excellent  Very good  Good  Fair  Poor  Don't know

**26) Have you ever had treatment for gum disease, such as scaling and root planing, sometimes called “deep” cleaning?**

Yes  No  Don't

**27) Have you ever had any teeth become loose on their own, without an injury?**

Yes  No  Don't

**28) Have you ever been told by a dental professional that you lost bone around your teeth?**

Yes  No  Don't

**29) During the past 3 months, have you noticed a tooth that doesn't look right?**

Yes  No  Don't

**30) Aside from brushing your teeth with a toothbrush, in the last 7 days, how many times did you use dental floss or any other device to clean between your teeth?**

\_\_\_\_\_ Number

**31) Aside from brushing your teeth with a toothbrush, in the last 7 days, how many times did you use mouthwash or other dental rinse product that you use to treat dental disease or dental problems?**

\_\_\_\_\_ Number

**32) Have you had periodontal disease with bone loss?**

Yes  No  Don't

**33) How many natural teeth do you have?**

None  1-10  11-16  17-24  25-32

**34) Are you currently suffering from recurrent ABDOMINAL pain or discomfort (including nausea) that has bothered you at least 3 days per month in the last 3 months?**

No  Yes (Please answer questions a-f)

(go to #35) a. When did the abdominal pain or discomfort begin?  Month  Year


b. When you have these symptoms, how often do they occur?  
 Daily  At least 3 times per month  Once a month  Less than once a month

c. Is your abdominal pain or discomfort better after defecation?


Appendix 9 – Stool and saliva microbiome questionnaire

Yes, always     Sometimes but not always     No     Not sure

d. Is the onset of abdominal pain or discomfort associated with a change in frequency of stool?

- Yes      I have FREQUENT bowel movements (>3 per day)  
 No     I have RARE bowel movements (<3 per week)  
 Not sure     I ALTERNATE between frequent and rare bowel movement over hours and days  
 I ALTERNATE between frequent and rare bowel movement over weeks and months  
 My bowel habits are in the range of <3 per day and >3 per week and do not change with time


e. Along with your symptoms and pain, did you have a change in the form (consistency and appearance) of stool?

- Yes      Hard and lumpy  
 No     Loose and watery  
 Not sure     Alternate between hard and loose stool

f. Does any of the following apply to you? (PLEASE CHECK ALL BOXES THAT APPLY)

- I recently lost weight (10 pounds or more) without trying  
 I feel abdominal pain or discomfort while I sleep  
 I have blood in stool on at least 25% of occasion  
 I have mucus in stool on at least 25% of occasion  
 I used antibiotic recently, name: \_\_\_\_\_  
 Family history of colon cancer  
 Physician diagnosed medical condition that can explain my gastrointestinal symptoms  
 Please specify the diagnosis \_\_\_\_\_  
 None of these apply to me

**35) Have you ever been diagnosed by a physician with IRRITABLE BOWEL SYNDROME (IBS)?**

- Yes        When do you think your symptoms of IBS started?   MM/YYYY  
 No    If not sure, please use approximate dates.  
  
When were you diagnosed with IBS?   MM/YYYY

**RETURN THIS DATA SHEET WITH THE SHIPPING BOX LABELED "SET #1"**