

## Collecting Urine and Saliva Samples

**Study participants:**

A very important part of this study is the collection of a 24-hour urine sample and the collection of 14 saliva samples (7 samples per day for 2 days). The instructions for the collections are attached. *Please follow these instructions very carefully.* If you deviate from the instructions for any reason, call [*study contact*] immediately so that she can tell you how to proceed. If you have any questions or problems, call [*study contact*].

**Thank you for your cooperation.**

## Collecting a 24-Hour Urine Sample

**Why do I have to collect urine for 24 hours?** The purpose of the 24-hour urine sample is to measure hormones that are involved in mental and physical activities such as exercise, stress, and working on tasks or problems. These hormones are called norepinephrine and epinephrine and are produced by nerves in the body and by the adrenal glands. They are important for several reasons and are a key part of this study. They will be used in much the same way as your blood pressure and heart rate readings to determine physiological activity. Please follow the instructions below very carefully. **YOUR URINE SAMPLE WILL NOT BE USED FOR DRUG OR ALCOHOL TESTING.**

**How do I collect the urine sample?** You will be given a jug that contains a caustic preservative (powder) in it. Do not open the container until you are ready to begin collecting the sample. Avoid contact with the powder as it can cause minor skin irritation. Each time you have to urinate, you should urinate in this container, making sure that you collect all the urine you produce. After each urination, screw the lid firmly back on the jug and place it in the refrigerator.

**Exactly when do I start and stop collecting my urine?** You will begin collecting your urine on Sunday, [date] and end the collection the following Monday morning. Begin collecting your urine after you go the first time on Sunday. In other words, when you get up on Sunday, go to the bathroom as usual and flush it away. From then on, save all of your urine in the brown jug. Do this for the rest of the day Sunday, Sunday night, and on Monday morning. You can quit collecting your urine on Monday at the same time as you started collecting it on Sunday. For example, if you started collecting your urine at 9:00 AM on Sunday, you should collect all of your urine up to and including any urine you can produce at 9:00 AM on Monday. This will make it a full 24-hour sample. Please provide as complete a sample of urine as possible.

**Are there any restrictions for the urine collection?** Yes! It is important that you do not take any cold or sinus medications (except Tylenol) after Saturday morning [mm/dd] at 8:00 AM. You may resume taking these medications after the urine collection is over on Monday, [mm/dd].

**Do I need to refrigerate the urine?** Yes. Samples should be refrigerated as much as possible during the collection period and until you return the sample to us.

**What is the "Urine Collection Record"?** This form has some questions that you need to answer after you've completed the urine collection on Monday. Because the hormones that we are looking at may be affected by how much caffeine you consume, how much you smoke, and how active you are, we need you to answer some questions regarding these things. The form also has space for you to discuss any problems that you had during the urine collection (see below).

**What if I miss a sample?** It is very important that you do not miss any samples! If you should miss a sample, record this on the Urine Collection Record. PLEASE TRY NOT TO MISS ANY SAMPLES, but if you do, we need to know why the sample was missed and what time this occurred. Also, it is very important that you do not add water or other liquids to the jug.

*Please put your study ID number and initials on the label on the brown jug.*

## Collecting Saliva Samples

**Why do I have to collect saliva samples?** The purpose of the saliva samples is to measure cortisol, which is a hormone involved in the metabolism of glucose. This hormone is important for several functions in the body and is a key part of this study.

**When do I collect the samples?** You will be collecting saliva samples on Wednesday, [mm/dd] and Friday, [mm/dd]. You will collect 7 samples on each day. We are lending you an alarm watch that will beep when it's time for you to collect a saliva sample. (The first sample of the day will be collected using a timer instead of the watch. This will be explained in more detail below.) It's very important that you collect the sample as close to the sound of the alarm as possible. See your own personalized schedule so you know when to expect the alarm to go off.

**How do I collect a saliva sample?** You will be provided with collection tubes called salivettes. Each salivette consists of a test tube with a smaller container inserted in it and a wedge of cotton similar to those used by your dentist. Please collect all saliva samples with these tubes. When it is time to take a sample, follow the steps below:

- Take the lid off of the tube containing the cotton wedge.
- Remove the cotton wedge and chew gently on it for about 2 minutes or until the cotton is soaked. You may simply place it between your cheek and gum, under your tongue, or roll it around your mouth. Please do not bite down on it hard or chew it vigorously. Your goal is to get the wedge as wet as you can.
- Place the cotton back into the tube and put the lid back on securely. (Be sure that the cotton is placed so that it is in the container in the upper part of the test tube.)
- Be sure to write: (1) your ID number, (2) the day and exact time that you took the sample, and (3) the CODE from the watch, all on each tube label.
- There will be a special code that appears when the alarm goes off. It will be a combination of numbers and letters, and it will appear on the top line of the watch's display. Please record this code on the tube label where it says "code".

- **IMPORTANT:** You must record the code displayed on the watch **BEFORE** you stop the alarm; when you stop the alarm the code will disappear.
- Place each completed sample in the extra storage bag and refrigerate.

**How do I operate the watch?** To turn the alarm off and get the regular display back on the watch, push the button on the bottom left side of the watch. You may have to push the button one or more times. You can't hurt any of the watch's settings by pushing this button, but please don't touch any of the other buttons on the watch. Your watch is NOT waterproof, please take care not to get it wet and do not wear it in the shower.

**Exactly when do I take the first sample of the day?** The timing of the first sample of the day is extremely important! It must be collected exactly one hour after you wake up. We know that it is difficult for you to know the exact time that you will wake up on Wednesday and Friday. Therefore, we are also lending you a timer that you will set in order to collect the first sample at precisely one hour after you awaken. The procedure is as follows. As soon as you wake up, record the current time on the sheet entitled "When to Take Saliva Samples." Also record the time that you should take the first sample (exactly one hour after you woke up). Then set the timer for one hour. When the timer goes off, take the first sample of the day and label the tube as instructed above. You will not have a code for this sample since you are not using the watch and that is where the code comes from.

**What do you mean by "wake up"?** By "wake up" we mean the time that you awaken and stay awake. So, for instance, say you wake up at 6:00 AM, then roll over and sleep a little longer. At 6:25 you wake up again, and this time you stay awake. Your wake-up time is 6:25 AM, not 6:00 AM.

**When do I take the rest of the samples of the day?** For samples 2 through 7, you will use the alarm watch that we have lent to you. It will beep at the times indicated in your personalized schedule. These times are based on the approximate time that you thought you would be getting up on these 2 days.

**What if I wake up at a different time than I said I would?** If the time that you awaken is no more than one hour before or one hour after the time you had originally given us, it's okay. Take the first sample of the day as described above, and use the watch to take samples 2 through 7. If the time that you awaken is more than one hour before or after the time you had originally given us, you will have to do the samples on a different day. Call [*study contact*] immediately so that she can tell you how to proceed. For your convenience, please try to wake up within this 2-hour interval!

**Are there any restrictions during the saliva sample collection?** Yes! It is important that you do not eat or brush your teeth within the hour (60 minutes) before you are scheduled to take a sample. Also, please do not smoke within 30 minutes before a sample, and no chewing gum within 10 minutes before a sample. If you forget and do eat, smoke, brush your teeth, or chew gum before a sample, please record that information in the space provided at the bottom of the Saliva Collection Record (see below). Please let us know exactly what you did and what time you did it.

**Do I need to refrigerate the saliva samples?** Yes. Please refrigerate the samples as soon as you possibly can (at least by the end of the day on which you collect them).

**What is the "Saliva Collection Record"?** The Saliva Collection Record is a form that has some questions that you need to answer after you've completed the saliva collection on each of the two saliva collection days. There is a Saliva Collection Record for Wednesday, and also one for Friday.

**What if I have a problem of any kind?** Call [*study contact*] immediately and she will tell you how to proceed.

*Please note that you will have to do the saliva samples over if codes are missing, samples were taken at incorrect times, etc. If you have any problems during the saliva collection, it is important that you call [*study contact*] immediately so that she can tell you how to proceed!*