

24-HOUR URINE SPECIMENS**1. Introduction**

The 24-hour urine samples obtained in this part of the examination will be used for future studies. Studies may examine the role of hormone concentrations (for example, melatonin and cortisol) as potential mediators of the association between sleep disorders and outcomes such as osteoporosis, cardiovascular disease, and decline in cognitive and physical function.

The objectives for this part of the examination are:

- a) prepare 24-hour urine collection containers;
- b) distribute urine collection containers and instruct participants in proper urine collection technique;
- c) process returned, completed urine collections.

2. Participants

Twenty-four hour urine collections will be obtained at the MrOS Sleep visit on the Portland clinic participants. Participants who are incontinent are excluded from the 24-hour urine collection, and should only do the first morning void collection. All participants that do the 24 hour urine collection will also collect their first morning void the morning after their PSG study in a separate container. Ask all participants if they lose significant amounts of urine (1 cup or more) on a daily basis. If they say yes, then they should be excluded from the measure.

3. Equipment

24-hour urine collection containers-4 liter size (Fisher Cat #14-375-115)-1 per participant
Small lab spatula (Fisher Cat #14-356-10)
Bottle crystal thymol preservative (Fisher Cat #T185-100)
Mineral oil, U.S.P. heavy-70cc per participant
Permanent ink, felt tip marker ("Sharpie"), black-(Sanford #30001)
Urine instruction sheet "How to Collect a 24-Hour Urine"
18 gauge, 6 in, blunt-tipped filling needle
60 cc syringes (B-D #301627)-1 per participant
50 cc polypropylene centrifuge tubes for shipping sample from 24-hour urine collection (Fisher Cat #14-959-49A)-1 per participant
Buffer solutions: yellow-pH 7.0 (Fisher Cat #SB107-500), and red-pH 4.0 (Fisher Cat #SB101-500)
Scale (Denver Instruments Electronic Top-loading Balance Model XL6100)
pH meter (Fisher Scientific #13-300-86)
Cryotubes for urine archiving 3.5 cc capacity (Applied Scientific #AS-2307)-2 per participant
Disposable plastic beakers for pH testing (Fisherbrand Polystyrene Beakers in Dispenser Carton Cat #02-544-37)-3 per participant

100 cc cylinder (Fisher Cat #08-572D)

4. Preparation of 24 hour Urine Containers

- 1-2 weeks supply of containers may be prepared at one time.
- Open the specimen container into a cube shape.
- Put on gloves. The thymol preservative can be irritating to skin. If you get some on your skin, wash it off thoroughly.
- Remove the cap of the container.
- Using the spatula, put a scant 1/2 spatula-full of thymol crystal preservative into urine container. Only a few grains are necessary.
- Pour 70 mls of Heavy Mineral Oil into the 100 ml cylinder. Then pour the mineral oil into the urine container.
- Replace the cap on the container.
- With nothing on the scale, press the button marked "zero." The scale will register "0.00." The scale is now ready. If the scale does not register "0.00," be sure there is nothing leaning on it, it is on a level surface, and out of drafts.
- Weigh capped urine container (with thymol and mineral oil inside) on the electric top-loading scale to nearest 0.1 gram.
- Write the weight on the outside of the urine container with a permanent-ink, felt-tipped marker ("Sharpie"). This is the "prepared weight."
- Graduated cylinder may be rinsed out with water and wiped dry with paper towels as needed.

5. Distribution of Urine Containers to Participants Collecting 24-hour Urines

- At the beginning of the visit, have participant empty bladder completely in bathroom. (This urine is not saved.)
- Write participant's MrOS ID # on a "How to Collect a 24-Hour Urine" instruction sheet.
- Record the time at which the participant urinated on the "How to Collect a 24- Hour Urine" instruction sheet for that participant.
- Review "How to Collect a 24-Hour Urine" instruction sheet with participant.
- Explain to participant you would like him to describe collection procedure back to you. Suggested text: "To be sure you understand how to collect your urine, I'd like you to

describe back to me how you're going to do the urine collection at home." Have participant describe procedure for the 24-hour urine collection to you. If correct, continue. If incorrect review instructions again until participant can describe procedure correctly.

- Write participant's MrOS ID # on a prepared 24-hour urine collection container.
- Give participant the prepared 24-hour urine collection container with their MrOS ID # written on it and the instruction sheet "How to Collect a 24-Hour Urine" with their MrOS ID # written on it.

QC Checklist for Distributing 24-Hour Urine Collection Containers

- Prepare container
- Have participant empty bladder
- Write time ppt emptied bladder on instruction sheet
- Review instruction sheet with ppt
- Use correct text to have ppt describe back urine collection procedure
- Give ppt container and instruction sheet

6. Procedure for Processing Completed 24 Hour Urine Specimens

A. WEIGHING RETURNED COLLECTION

- Twenty-four hour urine collections should be returned to the clinic within 48 hours of completion.
- Completed 24-hour urines should be processed as soon as possible upon return of the collection to the clinic. Urine collections should be kept refrigerated until processed. Non-sterile gloves should be worn during all procedures involving handling of urine.
- With nothing on the scale, press the button marked "zero." The scale will register "0.00." The scale is now ready. If the scale does not register "0.00," be sure there is nothing leaning on it, it is on a level surface, and out of drafts.
- Place full, capped container on scale. The number on the scale is the weight (in grams) of the container + thymol + cap + urine. This is the "filled weight."
- Record prepared weight (which is written on the container in permanent marker from when it was first prepared) and the filled weight (just measured in previous step) on the "24-hour Urine Collection" data collection form.

B. REMOVING NET ACID EXCRETION SAMPLE

- Using a permanent ink marker such as a "Sharpie," label the 50 cc polypropylene tube with the participant's MrOS ID number.

- Write the participant's MrOS ID number, height, and weight on a "24-Hour Urine" data collection form.
- Place a clean blunt ended 6 inch filling needle on the end of a 60 cc syringe. Draw up about 5 cc's of air into syringe.
- Open the 24-hour urine container. Place the needle end below the level of the oil, several inches into the urine itself, near the bottom of the container.
- Expell the air out of the syringe into the urine. This will expell any oil that may have gotten into the needle as it passed through the oil layer. Wait until the air has bubbled up out of the urine before proceeding.
- Draw up 60 cc of urine, being careful not to draw any oil into the syringe.
- Remove the needle and syringe from the urine container. Wipe off the needle with a gauze pad or paper towel. Remove the needle from the syringe. Push out any air in the syringe and cap the syringe. **It is important that the urine not come in contact with air before the pH is measured.**
- Place 45 cc of urine in the 50 cc polypropylene tube. Re-cap the syringe, again pushing out any air in the syringe so that the urine is not in contact with the air. Do not fill the 50 cc polypropylene tube above the 45 cc line as the sample will expand as it freezes. Screw the cap onto the polypropylene tube. The urine in the polypropylene tube is the Net Acid Excretion specimen. The urine remaining in the syringe will be used to determine the urine pH and for archiving.

C. DETERMINING THE URINE pH

- Set up three disposable beakers.
- Dispense approximately 10 cc of the yellow buffer solution (pH 7.0) into the first disposable beaker (level should be about 1/2 inch) and 10 cc of the red buffer solution (pH 4.0) into the second beaker.
- Place approximately 10 cc of the urine remaining in the syringe into the third disposable beaker. The urine still remaining in the syringe will be used for the archived sample.
- Remove the protective cap from the Fisher pH tester. Do not wipe the end of the pH tester dry. This will cause a build-up of static electricity and make the probe inaccurate. Turn the tester "on." The "on/off" switch is the top button on the back of the unit.
- In order for the pH meter to calibrate itself correctly, it must be placed into the two buffer solutions in the order specified below.
- Immerse the probe into the pH 7.0 buffer (yellow). Do not immerse the tester above the maximum immersion level marked on the tester. Press and hold the "on/off" button for three seconds, then release. The probe will flash "7.02" for about 15 seconds.

- After about 15 seconds, the probe will stop flashing and read "4.00." This is a prompt for you to place it into the pH 4.0 buffer (red). Place it into the red buffer. It will flash for about 15 seconds, then will stop flashing and read "4.00." At this point it is calibrated and you are ready to read the pH of the urine.
- Place the probe into the urine. Wait until the reading stabilizes (about 15 seconds).
- If the urine pH is less than 4.5 or greater than 9.0, re-calibrate the pH meter and read the same urine sample again. If the reading is between 4.5 and 9.0, record it on the data collection form. If it is still less than 4.5 or greater than 9.0, withdraw a fresh urine sample from below the oil layer with a new syringe and repeat the pH tester calibration and sample reading. Record this final reading on the data collection form even if it is still below 4.5 or 9.0.
- Record only the final urine pH value on the data collection form. Note that if the initial reading is between 4.5 and 9.0, this will be the only reading.
- Be sure to turn off the pH meter when done.
- For storage, when you are finished with the tester, place a few drops of pH 4.0 buffer into the protective cap of the electrode and replace the cap. Store upright so the probe is always wet.
- The batteries (4 x 1.4 volt batteries) should last a long time (300 hours), but if you should get an Eb error code, replace them. The unit has an automatic shutoff after 10 minutes, so if you forget to shut it off, it will automatically shut off.
- Clean the filling needle by running distilled water through it and drying upright. Before using it again, push air through it to get rid of any water that may remain inside.
- The pH tester is warranted for six months. For customer service, call Fisher Scientific at 1-800-766-7000.

D. ARCHIVING THE URINE SAMPLE

- Label two 3.5 cc capacity cryotubes with the participant MrOS ID #. Use a pen with permanent ink such as a "Sharpie." Keep the labeled cryotubes away from solvents such as alcohol or acetone as these will erase the ID code. Before transferring the urine, the 24-hour urine collection container and cryotubes should be held side by side and the numbers read aloud to check that the ID code numbers match. Do not set up production lines of labeled empty cryotubes. The chance of error is increased by the latter procedure.
- Using the urine still remaining in the 60 cc syringe, place enough urine into each of the two cryotubes to fill them up to the 3.5 ml mark. If there is not enough urine left in the 60 cc syringe to fill the cryotubes, withdraw another urine sample from below the oil layer using a fresh syringe and the technique described above.

- Some caution should be used in capping the cryotubes. Screw the caps on firmly to secure them tightly, but do not apply an extreme amount of pressure. All three urine samples (Net Acid Excretion sample in polypropylene tube and two cryotubes) should be frozen at -20°C as soon as possible (within 4 hours) to prevent growth of bacteria that could interfere with measurements. To promote rapid freezing, place the samples upright in a footless metal rack that is in contact with a shelf in a -20°C freezer.
- Mark the number of tubes archived on the data collection form.

QC Checklist for Handling Completed 24-Hour Urine Collections

- Weigh returned container
- Label polypropylene tube
- Draw 60 cc's of urine from near bottom of container
- Expell air from syringe and recap
- Place 45 cc's of urine into polypropylene tube
- Expell air from syringe and recap
- Set up three disposable beakers
- Place buffer solutions in first two beakers
- Place 10 cc urine in third beaker
- Calibrate pH tester and measure urine pH
- Label cryotubes
- Fill cryotubes to 3.5 ml mark

7. Temporary Cryotube Storage and Shipment

- After samples have been frozen by placing cryotubes upright on a -20°C shelf overnight, place cryotubes in ID numerical order into a storage box using the inserts.
- Use the cryotube storage box grid for recording the position of cryotubes, by ID number, within the shipping boxes sent to BRI. (This is a back-up identification system in case the ID numbers on the tube are obliterated after prolonged storage at -70°C .) As the filled tubes are placed into the slots formed by inserts, write the ID number which is on the tube into the corresponding box on the paper grid.
- Since the box does not have a definite up or down, right or left, you will have to mark the upper right corner of the cardboard box and the insert. (The paper grid is already marked "upper right" and "upper left.") Orient the box so that the oval holes along the bottom of the box are facing toward you on one side and away from you on the other side. In a clearly visible spot in the upper right corner of the box and the insert (to the right and away from you), punch a hole in the cardboard with a single hole paper punch.
- Store samples at -20°C in the storage box until 100 cryotubes have been filled and frozen.

- When the box is ready for shipping, record on the grid form the dates over which the samples were collected, your clinic, the date the box was shipped to BRI, and the number of tubes being shipped.
- Send one copy of the form to BRI with the box and keep one copy yourself.
- When the box arrives at BRI it will be assigned a unique identifier and placed into storage. A copy of the grid with the identifier will be sent to the coordinating center.
- A box of samples should be shipped in an insulated shipping box on dry ice by a 24-hour air carrier (such as Federal Express). To ensure that the samples can be received at BRI the next day, ship Monday-Wednesday only. The insulated shipping box and carrier can be chosen by each Center. However, the shipping box must provide insulation and have inner dimensions sufficiently large to handle 1 storage box (outside dimensions 5.25 x 5.25 x 4.75 inches) and 5-10 lbs of chipped dry ice to keep the samples cold even if shipment is interrupted for a day or two. (If in doubt, err on the side of too much dry ice.)
- Urine cryotubes should be shipped to:
Chris Kennell
Biomedical Research Institute
12264 Wilkins Ave.
Rockville, Maryland 20852