

2-hour urine protocol draft  
Revised 9/25/96

## PROTOCOL FOR IN-CLINIC TIMED URINE SPECIMENS

### 1. Introduction

In-clinic timed urine samples will be archived from all participants in V6 except the V12 cohort. The V12 cohort will have urine archived from their 24-hour urine collections. Archived urine will be used to determine kidney function and markers of bone loss.

### 2. Equipment

Non-sterile disposable gloves  
Urine collection hats-1 per participant  
250 cc graduated cylinder (Fisher #08-572-E)  
Brushes for cleaning graduated cylinder (Fisher #03-621-B)  
Pipettes (Fisherbrand disposable transfer pipettes #13-711-5A)  
Cryotubes for urine archiving 3.5 cc capacity (Applied Scientific #AS-2307)-2 per participant

### 3. Preparation of participants

Food changes the amount of calcium in the urine. Therefore, all participants must not eat anything for five hours before the collection. Water is permitted. If the participant breaks her fast before collection is complete, collect the urine and record the lack of fasting.

Participants taking insulin are excluded from this test.

To increase the amount of urine all participants should drink at least 16 oz of water within the first hour of the clinic visit. They should drink 8 oz as soon as they come to clinic and another 8 oz during the first hour of the collection period. There is no limit on the amount of water they can drink; this does not interfere with the tests.

### 4. Collecting the timed specimen

Timing is very important. The participant starts the collection time by completely emptying her bladder. This time should be recorded to the nearest minute on the data collection form. Enter the exact time on the "In-Clinic Timed Urine Collection" data collection form.

Non-sterile, disposable gloves should be worn during all handling of urine specimens. Urine will be collected in "urine hats" set in the toilet. **After at least 2 hours**, a staff member should place the hat in the toilet and the participant should go to the bathroom and empty her bladder into the hat. She does not need to clean her urethra and should not let any of the urine spill into the toilet. If urine is spilled, the collection should be restarted. After the participant has voided, a staff member should retrieve the hat and measure the urine by pouring it into the graduated cylinder.

A few participants may need to urinate more than one time during the timed collection period. Each time they urinate, the urine must be collected in a hat and the volume measured and recorded. Keep the urine from each void and combine at the end of the collection period. After at least 2 hours, participants should void for one final time and the collection period may stop. The

urine volume recorded on the data collection form is the total for all voids during the timed collection period.

The time at which the collection period ended must be recorded to the nearest minute on the "In-Clinic Timed Urine Collection" form. Figure the total number of minutes (at least 120 minutes) and enter on the form as "Total collection time." If a collection period lasts longer than 2 hours, that is fine; just be sure the exact amount of time is recorded on the form.

### **5. Measuring, freezing, and shipping the urine specimen**

The amount of urine voided should be measured in the graduated cylinder to the nearest 2 ml. The total amount collected during the collection period should be entered on the "In-Clinic Timed Urine Collection" data collection form. Most participants will have 50-200 mls. If a participant has more than 250 mls, measure the first 200-250 mls in the cylinder, write down the volume and discard this urine. Then measure the remaining urine and write the total volume on the data collection form.

For participants who void more than one time during the collection period, be sure the sample is taken after combining all the collected samples for that person.

Label two 3.5 cc capacity cryotubes with the participant SOF 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. Do not set up production lines of labeled empty cryotubes. The chance of error is increased by the latter procedure. Pipette enough urine into each of the two 3.5 cc capacity cryotubes to fill them up to the 3.5 ml mark.

If more than about 5 cc of urine is spilled in handling prior to taking the sample, throw out the urine and begin the collection period again.

The urine sample in the cryotube should be frozen at  $-20^{\circ}\text{C}$  as soon as possible (within 4 hours) to prevent growth of bacteria that could interfere with measurements. The urine tubes should be labeled, stored at  $-20^{\circ}\text{C}$ , just as you do with the serum, and shipped with regular shipments to BRI.

Rinse the cylinders with water several times between uses in a single day. Scrub the cylinders in a fast acting cleaner and solvent, such as Kler-Ro powder, at the end of each day.

### **QC Checklist for Timed Urine Collection**

- Participant empties bladder
- After at least 2 hours, place hat in bathroom
- Participant voids into hat
- Urine volume measured in graduated cylinder
- Cryotubes labeled
- Cryotubes filled to 3.5 ml mark