

SECTION 8. CLINICAL CENTER SAMPLE COLLECTION AND HANDLING

8.1 Laboratory Responsibilities

- A. The Central Biochemistry Laboratory at the Cleveland Clinic foundation will receive and perform all testing and reporting of results for the SMA-18, lipid profile, and 24-hour urine as indicated in Table 18.1 and 18.2 of the Protocol.
- B. The clinical center local laboratories will perform all of the testing and reporting of results for the CBC, urinalysis, and serum HCG.
- C. The clinical center AASK technician will be responsible for collecting all samples, processing them and mailing them to the Central Biochemistry Lab or sending them to the appropriate clinical center lab.

Table 8.1 Central Biochemistry Laboratory/Visits Procedures

Visit	SV1	SV2	G1	FV0.1	FV3	FV6 and Every 6 Months	FV12 and Annually
SMA18	2mls optional	2mls	2mls				2mls
Lipid Profile			4 mls				4 mls
24-hour Urine			25mls			25mls	
Serum Potassium and Creatinine				2mls	2mls	2mls	
Buffy Coat			X				
Serum After-Thought			4mls				4mls
Total Serum	2mls optional	2mls	10mls	2mls	2mls	2mls	10mls

Please note a 9.5 ml serum separator tube, when filled, will yield approximately 4mls of serum.

X - Buffy Coat-take thin white layer (leukocytes, white blood cells) between plasma & red blood cells
 -use 10 ml EDTA purple top

Table 8.2 Central Biochemistry & Local Labs

	10ml Red Top or 9.5ml SST (Red/Gray Speckled Top)	10ml (EDTA) Lavender Top for Buffy Coat	EDTA Lavender Top (Ask for CBC Local Lab for Size)	24-Hour Urines	Urinalysis	Serum HCG if Necessary
SV1	1 optional					
SV2	1		1		10mls	
BT1						
BT2						
G1	2	1	1	25mls		3 drops
G2	1					3 drops
CV						
FV0						
FV0.1	1		1			
FV1						
FV2						
FV3	1					3 drops
FV5						
FV6 and every 6 months	1			25mls		3 drops
FV12 and annually	2		1		10mls	3 drops

8.2 Telephone/Written Communications

- A. Telephone inquiries having to do with aspects of sample collection/processing/mailing may be directed to the Central Biochemistry Laboratory. Calls to the Central Biochemistry Laboratory will be answered between 8:30 a.m. and 5:00 p.m. Eastern time, weekdays only.
- B. Written inquiries can be sent to the Central Biochemistry Laboratory at the addresses in the AASK Address Directory.
- C. The Central Biochemistry Lab will communicate with participating clinical centers over electronic mail, or at the addresses/phone numbers listed in the AASK Address Directory.

8.3 Central Biochemistry Laboratory

8.3.1 Procurement of mailing supplies for biochemistry samples

The Central Biochemistry Laboratory will provide all necessary mailing supplies to the clinical centers for mailing of Biochemistry samples. This includes styrofoam insulated mailing containers with cardboard outer mailing boxes, 30 ml polypropylene urine mailing tubes, 15 ml polypropylene serum mailing tubes, zip-lock plastic bags, freezer packs, packing tape, and suitable mailing labels.

Participating clinical centers will be expected to provide sample processing supplies (e.g., tubes, needles).

8.3.2 Distribution of mailing supplies to participating clinical centers

Mailing supplies will be shipped to each participating clinical center, as needed. These supplies will be returned to the participating clinical centers by the Central Biochemistry Lab shortly after each set of patient samples is received. Styrofoam mailing containers and freezer packs will be reused whenever possible and replaced by the Central Biochemistry Lab as needed. Plastic sample mailing tubes and zip-lock bags are discarded by the Central Biochemistry Lab after each mailing and replaced. Supply order forms will be sent back to clinical centers with each remailing of shipping boxes. Clinical center technologists may check off needed supplies on these forms and return them to the Central Biochemistry Lab with the next sample mailing to indicate their need for additional supplies.

8.3.3 Postal inquiries

Participating clinical centers should keep a log of sample mailing dates for reference. The Central Biochemistry Lab will log-in samples received. In the event that mailing difficulties occur, the Central Biochemistry Lab will follow-up as needed when notified of a problem.

8.3.4 Sample package receipt at CCF

Samples will arrive as next-day-mail at the Cleveland Clinic Foundation (CCF). Packages are delivered directly to the Central Biochemistry Lab.

8.3.5 Communication with participating clinical centers concerning sample receipt problems, protocol changes, etc.

The Central Biochemistry Lab will communicate sample receipt problems, protocol changes, and other information to the participating clinical center personnel as needed.

8.4 Sample Handling at the Clinical Centers

8.4.1 Sample requirements

Table 8.1 lists the total volume of serum in ml needed to complete the various biochemical assays. In general, 10 to 20 ml of blood will be required (1-2 serum separator tubes). The table includes tubes and volumes for biochemistry assays done at the Central Biochemistry Laboratory. The volume indicated does not allow for rechecking any tests. Save, if available, 2 mls of serum for back up.

Draw extra serum for duplicate quality control samples for the CBL when required. Refer to this table for amount of serum needed. Extra urine aliquots are also needed for the CBL duplicate quality controls.

The sample requirements for the tests to be performed by the local laboratories may vary among the clinical centers and should be determined for each site. Table 8.1 of the manual may be copied and posted in your blood drawing area for easy reference. There are lines on it which can be filled in with the amounts required for local laboratory tests.

8.4.2 Labeling

All mailing tubes should be labeled with the participant's number, name code, date, and visit number. Urine aliquots must also have the total volume on the label.

Use of waterproof pen to write on the label or cover the label with waterproof tape to ensure that the writing will not smear. Double check to be sure the numbers and letters are clear and legible.

8.4.3 Instructions for drawing blood for biochemistry samples

The patient should be instructed to avoid medications listed in Appendix 19 for at least 2 days preceding a blood sample. The subject should be seated during venipuncture. Blood should be drawn from an antecubital vein, or failing this, from some other convenient arm vein. Hot packs (hot towels wrapped in absorbent pads) may bring up veins when none are apparent. The best policy is to take time to choose a good site initially. A tourniquet (e.g., an 18 inch length of Davol #9794 one-inch diameter Penrose drain tubing) or blood pressure cuff should be released prior to withdrawal of the blood sample, and at no time should it be left on for more than 3 minutes. Blood is drawn using the Vacutainer system following the instructions supplied with the system. A one-inch, 21 gauge needle is suggested. The needle should be placed in the vein. The required number of Vacutainer tubes should be filled as completely as possible. If, for some reason, a tube is not completely filled, an extra tube of blood should be drawn and combined specimens sent. All biochemistry samples must be drawn prior to ¹²⁵I-sodium iothalamate injection.

8.4.4 24-hour urine collection instructions

A preservative must be added to all 24-hour urine jugs before collection. The preservative for the urines is 5% acetic acid, 250 ml in every 4 liter urine jug. If a 4 liter jug is not available, a 1 gallon jug may be used with 250 ml preservative. The use of smaller containers for the 24-hour collection, is discouraged.

Use of prepared 5% acetic acid is recommended. However, one can make 5% acetic acid as follows:

1. Use a graduated cylinder of 250 ml or greater volume.
2. Put about 200 ml of distilled water into the cylinder. DO NOT USE TAP WATER.
3. Add 12.5 ml of reagent grade glacial acetic acid. USE CAUTION, THIS IS STRONG ACID. ALWAYS ADD ACID TO WATER.
4. Add water up to the 250 ml mark on the cylinder.
5. Mix well.
6. Transfer to a tightly sealed bottle or urine jug. This solution may be made up well in advance and stored in the urine jugs as long as they are kept tightly closed. In the patient education materials, there is an instruction form for collecting 24-hour urine. Use this to provide instructions to the patient.

8.4.4.1 Instructing the patient on collecting their 24-hour urine

1. Review with the patient the 24-hour urine collection instructions in Section 8.9.
2. Make sure they clearly understand the following points:
 - a. The liquid in the jug is a preservative, and must always be present in the jug. The preservative must not be discarded or washed out of the jug.
 - b. The first urine sample is not saved, but the time of this first urination is the beginning time for the collection.
 - c. Every urine sample during the 24-hour time period must be saved.
 - d. The patient must try to urinate 24-hours from the time the collection was started. This urine must be saved. If they cannot urinate at this time, this time is still used as the ending time.
 - e. If possible, the jug should be kept in a refrigerator during the collection period. Extreme temperatures are to be avoided.
3. Discuss with the patient which day during the month the collection should be started, and when the urine should be brought to the clinic. The patient should not be menstruating during the collection. If menses are irregular and occur at the time scheduled for collection, collection should be postponed until after the period is finished.
4. Discuss with the patient the importance of collecting all of their urine during the 24-hour time period. Let them know that it is not the quantity of urine that is important. What is important is that every drop of urine is saved. The test results will then accurately reflect what their kidneys are doing.
5. The patient should be instructed to avoid medications listed in Section 8.8 for at least 2 days preceding a 24-hour urine collection.

8.4.4.2 Acceptance of 24-hour urines

1. The 24-hour urine checklist (Section 8.10) should be completed by the technician when the urine is brought to the clinic. If this is not possible, one of the other members of the study team should take on this task.
2. If any "incorrect procedures" are checked off the list, the urine should not be sent to the CBL for analysis. It is better to have missing data than incorrect data in the database. The collection should be rescheduled as soon as possible.

The following criteria must be met:

- a. The urine jug should have contained the acid preservative. In rare instances, the preservative may be added when the urine is brought to the laboratory, provided that it is brought in on the day the collection is completed.
- b. Starting and ending date and time must be confirmed. The urine collection time must be

within 23.0 and 25.0 hours.

- c. The patient should have emptied their bladder at the "start time" and discarded this sample.
 - d. Every urine sample during the 24-hour time period must have been saved.
 - e. The patient should have emptied their bladder at the "ending time" and saved this urine.
 - f. The patient should not have had a short term illness during the collection.
 - g. The patient should not have been menstruating during the collection.
 - h. The patient should have drank the usual amount of fluids and eaten the usual amount of food during the collection period.
 - i. The jug should not have been frozen or overheated. Refrigeration during the collection is recommended.
3. If the technician discovers any information that may indicate that the urine was improperly collected, they should write this information on the checklist. The completed checklist is filed in the patient's file.
 4. Any questions about 24-hour urine collections should be directed to the CBL for clarification.

8.4.5 Processing the 24-hour urine samples

1. Tighten the urine container lid and mix the sample well (invert container thoroughly at least 5 times) to evenly distribute the acetic acid preservative and other components that may have settled upon standing. If two containers were used to collect urine, the urine in each container must be thoroughly mixed; then the two must be thoroughly mixed together before the aliquot is taken off. A large (2 gallon) container will be needed to mix larger volumes of urine.
2. Measure the total volume of urine including amount of acid, but read below any foam which may be present. Look at the cylinder at eye level to read the correct total volume. Record volume on the tube and record identifying data and urine volume on Form # 23. **DO NOT SUBTRACT THE AMOUNT OF THE ACID.**
3. Pour an aliquot of urine into a 30 ml urine mailing tube. If the technician is unsure about his or her ability to pour this without spilling, a pipette with a bulb can be used.
4. Close the lid tightly.
5. Fill out Form # 23 and mail with aliquots of urine.
6. Urine must be received at the CBL within 8 days from the start of collection.

7.Store 25 ml aliquot locally. This may be discarded once sample has been analyzed by central laboratory.

8.4.6 Serum for biochemical analysis

1.Record identifying data on Form 22.

2.Allow the blood to clot at room temperature for at least 30 minutes.

3.Spin down in a bench-top centrifuge.

4.Pour or pipet the serum into mailing tubes. Serum for any combination of biochemistry tests is sent in a single 15 ml red top mailing tube. Refer to the table (Section 8, page 2) for clarification of amount needed at each visit.

5.Close lids tightly.

6. Serum must be received at the CBL within 8 days from the draw date.

8.4.7 Clinical center local laboratory specimens

1.Record identifying data on Form 13.

2.If a urinalysis is required review with the patient the procedure for collecting a mid-stream urine sample (See Section 8.11).

3.Obtain blood samples according to the requirements of the local laboratory.

8.4.8 Instructions for Buffy Coat Collection

1.Collect one 10 ml EDTA purple top tube.

2.Mix thoroughly.

3.Spin tube 10 minutes in a bench top centrifuge.

4.Discard plasma, as much as you can without disturbing the Buffy Coat - the thin white layer.

5.Extract the Buffy Coat with as little contamination as possible from the plasma and red cells and place in the Nunc tube.

6. Label the tube with the patient ID, acrostic and visit number.

7 Freeze the sample (0°C).

8. Send to the CBL on an ice pack.

9. Please note: Sample must be processed same day, as soon as possible.

Buff Coat Tube Labeling Instructions

Label the orange labels in the following way:

ID number
Namecode (acrostic)
Visit #
Buff coat

Example: 012345
ROBGE
Visit #
Buff coat

Here's how to put labels on properly:

1. Pull back flap but leave attached.
2. Place patient information on bright orange sticker according to example above.
3. Peel entire sticker off white backing.
4. Wrap sticker around 1.8 ml Nunc tube, making sure you do not overlap any patient information.
5. Remove white piece of paper from clear tape.
6. Wrap clear tape around tube over orange label.

8.4.9 Sample storage

All CBL specimens (serum and 24-hour urine aliquots), should be mailed the same day, if possible.

The only exceptions to this are samples drawn or collected the day prior to weekends or holidays. Refrigerate these samples over the weekend; do not freeze them. These samples must not be mailed until Monday, since there is no one available to refrigerate the sample in the Cleveland Clinic Foundation mail room on weekends. Samples drawn late in the day should be processed the same day and mailed the following day. All filled mailing tubes should be refrigerated until they are packed with freezer packs in the styrofoam mailers.

8.4.10 Sample mailing instruction

Make sure to send all CBL samples and accompanying forms to the CBL lab. Place all biochemistry samples in a zip lock bag. Place two paper towels in these bags to absorb any leakage that might occur. These bags should be flattened by hand to remove air and sealed and then placed with one frozen freezer pack and placed into the styrofoam mailing container. Study forms (Form 22 or Form 23) may be placed into the styrofoam box, in which case they should also be in individual Ziplock bags to protect them from sample leakage and/or condensation from the freezer packs. A better approach is to include the paper work in the mailer by laying the forms (unfolded) on the top of the styrofoam box. No ziplock bag is needed in this case. The lid is put on, and the styrofoam box is slipped into the cardboard outer mailing box. This box is sealed with packing tape. All CBL samples should be sent by a next day mail service to the CBL address. Do not send samples so that they would arrive on a weekend, i.e., do not ship on a Friday.

8.5 **Abbott Testpack HCG-Combo Pregnancy Test Instructions**

1. Remove testpack from wrapper.
2. Fill sample pipet to first indentation, with serum.
3. Pipet serum into filter. Allow to soak in.
4. Add 3 drops of Reagent A. Wait 2 minutes.
5. Remove filter and discard.
6. Add 1 dispenser full of Reagent B. Allow to soak in.
7. Add 3 drops of Reagent C. Wait 2 minutes.
8. Add 1 dispenser full of Reagent D. Allow to soak in.
9. Read (+) as Positive, (-) as Negative.
10. Keep appropriate records of all patient results.

NOTE: This kit has been approved by the AASK Steering Committee for serum only.
Urine samples are unacceptable.

8.6 **Quality Control of the Abbott Testpack HCG-Combo Pregnancy Test**

Serum controls must be run to verify that the kit is working properly. Controls do not have to be run with each sample.

The recommended minimum frequency that controls must be run is once per week; if there are one or more patient samples to run that week. Controls may be run with each patient sample if the technician feels more confident about the test results. Remember to take into account the number of controls run when ordering kits.

8.7 Storage of Kit

Reagent A and serum controls must be stored refrigerated at 2-8°C. It is not necessary to refrigerate the entire kit, although refrigeration will not harm the kit in any way. The technician may find it easier to refrigerate the entire kit and controls, so everything is kept in one location.

The kit may be used up until the expiration date on the outside of the kit box.

NOTE: All reagents must be at room temperature prior to use.

8.8 List of Some Common Medications That You Cannot Take 2 Days Before a 24-Hour Urine Collection Test or Serum Measurements

Pain Medications

TRADE (brand name)	GENERIC NAME
Anacin	aspirin(acetylsalicylic acid)
Arthropan	salicylate salts
Bufferin	aspirin
Clinoril	sulindac
Dolobid.....	diflunisal
Empirin.....	aspirin
Feldene	piroxicam
Indocin.....	indomethacin
Medipren	ibuprofen
Motrin.....	ibuprofen
Naprosyn	naproxen
Various manufacturers	oxyphenbutazone
Rufen	ibuprofen
Disalcid	salsalate
Zorprin.....	aspirin
Advil.....	ibuprofen
Anaprox	naproxen sodium
Ecotrin	aspirin
Excedrin	acetaminophen/aspirin
Nalfon.....	fenoprofen calcium
Meclomen	meclofenamate sodium
Nuprin	ibuprofen
Butazolidin.....	phenylbutazone
Ponstel.....	mefenamic acid
Soma Compound	carisprodol/aspirin

Tolectin tolmetin sodium
 Voltaren..... diclofenac sodium

Antibiotics

Anspor cephradine
 Ceclor cefaclor
 Keflex cefalexin
 Velosef cephradine
 Duricef..... cefadroxil
 Ultracef..... cefadroxil
 Cefadyl cephalirin
 Keflin..... cephalothin
 Ancef ceftazidime
 Kefzol..... ceftazidime
 Mandol cefamandole
 Mefoxin..... cefoxitin
 Zinacef..... cefuroxime
 Monocid cefonicid
 Precef..... ceforanide
 Cefotan cefotetan
 Claforan..... cefotaxime
 Cefizox ceftizoxime
 Cefobid..... cefoperazone
 Moxam moxalactam
 Rocephin ceftriaxone
 Fortaz ceftazidime
 Tazidime ceftazidime
 Tazicef..... ceftazidime
 Suprax cefixime

ANTI-ULCER AGENTS

Alka-Seltzer antacids/aspirin
 Tagamet..... cimetidine
 Axid..... nizatidine
 Pepcid..... famotidine
 Zantac..... ranitidine

Urinary Tract Anti-Infectives

Bactrim..... trimethoprim/sulfamethoxazole
 Septra..... trimethoprim/sulfamethoxazole
 Co-Trimoxazole trimethoprim/sulfamethoxazole
 Cotrim trimethoprim/sulfamethoxazole
 Trimplex trimethoprim

Proloprimtrimethoprim

All nonsteroidal anti-inflammatory agents (including aspirin), cimetidine, ranitidine, trimethoprin/sulfamethoxazole, and trimethoprim must be withheld prior to a 24-hour urine collection or a blood test. This list only includes examples.

8.9 24-Hour Urine Collection

You will need:

Container for urine collection given to you in the Clinic. The liquid in the jug is a preservative and should not be discarded or washed out.

The day before you bring urine in:

1. Start your urine collection preferably in the morning. Void, do not save this sample but write the time and date on your jug.
2. Save every bit of urine all day and all night. Put it in the container.

The day you bring the urine in:

1. At the time noted the day before, void and save this urine. This completes your 24-hour collection. Write the time and date of completion on your jug.

This finishes your 24-hour collection.

2. Bring container to: _____

Things you will need to know:

1. It may be helpful to urinate in a clean tin can with a wide opening. Bend one side so you can pour from it.
2. Keep the jug of urine in a refrigerator, if possible. Avoid exposure of the urine to extreme temperatures.
3. Drink only as much liquid as usual.
4. If you're going to have a bowel movement, first pass your urine and save it so none will be lost.
5. Close the container securely and carry upright so it will not spill. A shopping bag makes this easier.

If you have any questions, telephone: _____

8.10 24-Hour Urine Collection

24-HOUR URINE CHECKLIST

Patient's Name _____

Date _____ Visit _____

When the patient returns a 24-hour urine, ask the patient the questions underlined below. The notes after each question indicate the correct procedures and should **not** be read to the patient. Check one box for each of question 1-9. The criteria for completing question 9 are found in the Manual of Operations.

Patient Followed:

Correct Procedures	Incorrect Procedures
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_____ 1. Did the jug contain any liquid (even a small amount) before you started collection? Note: The jug should have contained a small amount of liquid preservative. The patient should have kept the preservative in the jug.

_____ 2. At what date and time did you begin and end the collection? Note: There should be no less than 23 hours and no more than 25 hours between the start and end of the collection. The dates and times stated should match those on the jug.

_____ 3. How did you start the collection? Note: The patient should have emptied his/her bladder, discarded the urine, and marked the time and date on the jug.

_____ 4. How did you proceed with the collection? Note: The patient should have saved urine in the jug every time he/she emptied his/her bladder and not spilled or splashed any urine out of the jug.

_____ 5. Were there any times when you did not save urine in the jug? If so, how many times?
Note: The patient should have saved every urine.

_____ 6. Was there anything unusual about the day of the collection? The patient should have drank the usual amount of fluids, eaten the usual amount of food, not felt ill (including cold, flu, any illness or infection), and not have been menstruating.

_____ 7. How did you end the collection? Note: The patient should have emptied his/her bladder, saved the urine in the jug, and marked the time and date on the jug.

_____ 8. Where did you store the jug after ending the collection? Note: If possible, the jug should have been stored in the refrigerator. The jug should not have been frozen or overheated such as when stored in a car during the heat of summer or in below freezing temperatures.

9. _____ Urine is acceptable _____ Urine is unacceptable

COMMENTS

8.11 Mid-Stream Urine Procedure

1. Wash hands with soap and water.

2. Remove towelettes. Do not remove jar. Cleanse perineal area with all towelettes.

Females: Separate folds of urinary opening with thumb and forefinger and clean inside with towelettes using downward strokes only; keep separated during urination.

Males: Clean head of penis.

3. Remove container. **DO NOT TOUCH INSIDE OF CONTAINER.**

4. Begin urination into toilet. As urination continues, bring container into stream. **FILL CONTAINER ONLY HALF WAY.**

5. Remove cap from package with thumb and forefinger. **DO NOT TOUCH INSIDE OF CAP.** Screw cap on container and affix label.