SECTION 7. MEASUREMENT OF GLOMERULAR FILTRATION RATE

7.1 Introduction

The primary outcome variable, upon which the results of the full-scale trial will be based, is the rate of change in GFR. Therefore, measurement of GFR is a critical function of the full-scale trial. Each of the participating Clinical Centers has a GFR technician whose primary responsibility is the accurate measurement of Glomerular Filtration Rate, or GFR.

Glomerular filtration rate (GFR) will be measured at each Clinical Center by a trained technician at the following time points during the study: 1) entry into blood pressure titration period (G1); 2) pre-randomization (G2); 3) at 3-months and 6-months post-randomization; 4) every 6-months thereafter; 5) at any stop point; 6) after a potential GFR action item; 7) at the end of the study.

The Central GFR Laboratory at the Cleveland Clinic Foundation will implement and coordinate the glomerular filtration rate (GFR) procedures for measurement of GFR, including GFR sample receipt and counting, GFR calculation, and result reporting.

The Clinical Centers will be responsible for performing the GFR test and shipping the processed samples together with the GFR procedures form (#24) to the Central GFR Laboratory.

7.2 Clinical Centers' Responsibilities

7.2.1 GFR Test Protocol

The trained GFR technologist will perform the required GFR tests according to the test protocol. The training at the Central GFR Lab at the Cleveland Clinic Foundation will insure a common understanding of the testing technique. If a Clinical Center technician needs advice during a test, he or she should call the GFR Lab for advice on how to proceed. Written explanations of any deviations from the test routine should be included on the GFR worksheet.

The Glomerular Filtration Rate Protocol is as follows:

Principle

Urinary clearance of the GFR marker, Glofil (^{125}I-Iothalamate), after subcutaneous injection will be used to determine accurately the level of glomerular filtration in subjects with renal insufficiency by a method independent of changes in lean body mass or changes in protein intake. The patient ingests an oral water load, is given a saturated solution of potassium iodine (SSKI), and the Glofil is injected subcutaneously. After a 60-90 minute waiting period, timed collections of urine and serum are performed. GFR is equal to the urinary clearance of the marker.

Materials and Equipment

1.To perform GFR
a.Saturated solution of potassium iodide (SSKI)
b.Scale to weigh patient
c.Drinking cup and pitcher of water
d.Accurate timing device (digital clock and/or stop watch)
e.Urine collection containers (paper cups with lids, speci-pans or "hats" for females, urinals for males)
f.Graduated cylinder to measure urines
g.Blood drawing supplies (needles, syringes, tubes, alcohol wipe, gauze, a tourniquet or a blood pressure cuff, 0.9% saline, heparin-1,000 unit/ml, paper tape, band aid, and any other supplies)
h.Dose of Glofil
2.To process samples:
(Only use equipment designated for radioactive specimens)
a.Refrigerator to store samples
b.Centrifuge
c.Tubes to store backup duplicate samples at the center
d.Mailing supplies supplied by the Central GFR lab (labels, tubes, zip-lock bags, mailers, ice packs, and packaging tape).

Procedure

1.Check eligibility. The patient has been fasting for 8 hours. The patient cannot have anything by mouth except water for 8 hours prior to the test. The technician should verify this with the patient to uncover the patient who forgot to fast. In the unusual circumstance that the patient cannot tolerate an 8-hour fast, or if the procedure must be performed in the afternoon or evening, the procedure may be modified as follows: the procedure is performed after a 2-hour fast. During the 2 hours prior to the 2-hour fast, the patient should not drink caffeinated beverages, but may eat a light meal, containing < 2 gram protein (See Section 7.8). Please note that every effort should be made to perform all GFR procedures on an individual patient under the same conditions each time, that is at the same time of day and after the same duration of fasting. This means that once a patient has consumed the light meal prior to the GFR test, it will be necessary to begin with a similar meal before each and every following GFR test. Non-steroidal anti-inflammatory agents, including aspirin and ibuprofen will be withheld for at least 48 hours prior to each GFR test. Other drugs should be taken up to and including the morning of the GFR test as usual.

Women of childbearing potential (post-pubertal, premenopausal, and not surgically sterilized) **must have** a qualitative serum pregnancy test (HCG-RIA) within 72 hours prior to

the GFR determination. The GFR is to be canceled if the test was positive or if the patient did not have the test. If the serum pregnancy test is done outside of the Clinical Center or at the Clinical Center, written results must be on file, otherwise an Abbott Testpack HCG-COMBO Serum Pregnancy Test must be done on the morning of the GFR. Complete instructions for the Abbott pregnancy test can be found in Section 7.10.

If the patient has had a radionuclide diagnostic test in the past month, using an isotope other than ^{99}Technetium or ^{125}I, the GFR must be rescheduled.
(Any patient with a true iodine allergy is excluded from the Study.)

2.The patient should have had a 5 ml/kg water load at home.

3.Record the patient's height (cm) and weight (kg).

4.Mix 5 drops of SSKI in 20 ml of water and give to the patient orally. Record the time given. (The SSKI prevents thyroid uptake of any free ^{125}I. This protects the patient and eliminates error in the GFR determination due to the additional elimination route for the isotope).

5.Start hydrating the patient. A water load of 10 ml/kg is required given during the next 90 minutes. See "Procedure Notes," items 1 and 2 of this section.

6.Collect a background urine; record the time.

7.Draw the background blood sample and the appropriate Biochemistry samples after inserting the heparin lock, but before the Glofil injection. The recommended procedure for drawing GFR samples is as follows:

a.The subject should be seated during venipuncture.

b.It is recommended that the blood be drawn from an arm vein using an I.V. catheter/needle unit with a heparin lock. A recommended set is a 22 ga. 1 in. Insyte-W (Becton-Dickinson vascular access, cat. no. 3884221) and 6" catheter extension set (Baxter Interlink, cat. no. 2N3374 with the injection site removed, and Vacutainer tube holder with a screw-on leurlock adapter (Becton-Dickinson cat. no. 367290). The 6-inch line allows free motion for tube changes.

c.Insert the I.V. catheter into the vein. Draw all the biochemistry samples and the GFR baseline sample. Immediately after obtaining the samples, heparinize the site with at least one ml. of 100 u/ml heparin. See "Procedure Notes," item 5 of this section.

d.After heparinization, the line may be re-capped. The needle should be taped down securely with paper tape or some other easily removed hypoallergenic tape. Avoid taping the needle down to an extreme degree; this may pinch off the flow of blood.

8. At least 30 minutes following the administration of the SSKI, the Glofil is injected subcutaneously in the upper arm. Record the time. See "Procedure Notes," item 6 of this section.

9. Continue to hydrate the patient at the rate of 200-400 ml/hour as tolerated throughout the study.

10. At least 60 minutes after the time of the Glofil injection, the patient should void. Record the time (time #0). Measure the urine volume. See "Procedure Notes," item 3 of this section. Determine the difference in time between the collection time of the background urine sample and time #0. Divide the volume of the urine by this time difference. If this flow rate is at least 3 ml/min., then continue with the test (go to Step #11 immediately below). If it is less than 3 ml/min., wait a full 90 minutes from the time of the Glofil injection. Have the patient void again. Record the time. Pool both urines to determine the volume of the discard urine. Using the latest time and the time of the background urine calculate the flow rate and if the flow rate is $\geq$ 3 ml/min, continue with the test. If the flow rate is still inadequate, try another discard at 120+ minutes. If, after 120+ minutes, the flow rate is $\geq$ 2 ml/min, continue with the test. If not, call the Central GFR Lab for advice.

11. Draw a blood sample (S-#0--7 cc SST red/gray tube) using the heparin lock. To draw GFR samples, use two tubes for each draw. With the first tube, draw 2 ml. of heparin/blood to avoid diluting the GFR sample. Discard this initial diluted sample; then draw the GFR sample in the second tube. Always re-heparinize promptly. The heparinized solution may be used repeatedly for the same patient, but be sure to keep the syringe capped between draws.

12. After a minimum of 30 minutes (or more, depending on the ability of the patient to void), collect the next urine sample (U-#1). Record the time. Measure the volume. (If the flow rate for the period is low, extend the period to get additional urine and a higher flow rate, as described in #10 above). the flow rate for urines U-#1 through U-#5 is required to be $\geq$ 1.0 ml/min.

13. Draw the next blood sample (S-#1). Reheparinize the line.

14. Repeat steps 12 and 13 until four timed urines have been collected and appropriate blood samples drawn.

15. When all the blood samples have been obtained, remove the I.V. catheter. Have the patient apply moderate pressure at the site for five minutes to avoid bleeding. Then apply a bandage.

7.2.2Preparing the Samples for Mailing to the Central GFR Lab

1.When the blood samples have clotted, centrifuge the blood samples.

2.Be sure to include the patient's name code and number on each of the pre-labeled tubes.

3.Place half of the serum in an appropriately labeled tube; a minimum of 1 ml must be sent. Save the rest as a backup sample in the refrigerator. (Discard the duplicate when GFR results are received unless you are asked to submit the backup sample labeled with the QCID for quality control of the Central GFR Lab).

4.Place an aliquot of measured urine in the appropriately labeled tube; a minimum of 1 ml must be sent. Save a duplicate sample in the refrigerator. (Discard the duplicate when GFR results are received.)

5.Tighten all the caps of the mailing tubes.

6.Prepare the mailer for shipping with frozen ice packs.

7.Place all GFR tubes in a zip-lock bag. Place a paper towel in the bag to absorb any leakage that might occur. Attach a piece of yellow/magenta tape with a message "radioactive" on the outside of the bag. These bags should be flattened by hand to remove air and sealed.

8.Check the appropriate GFR form to make sure that it is filled in completely. Include the form in the mailer by laying it folded on the top of the inner styrofoam box.

9.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 Central Lab GFR samples should be sent by a next-day mail service to the GFR Central Laboratory address:

AASK Central GFR Lab, Desk A101
Cleveland Clinic Foundation
East 102nd Street
Cleveland, Ohio 44195

PROCEDURE NOTES

1.A well-hydrated patient is crucial to a valid GFR study. While 200-400 ml/hour is the recommended water load, the technicians should use their own good judgment based on the patient's kidney function to determine the appropriate amount of hydration. A desirable flow rate is 4-6 ml/minute, but this may not be achievable in some patients. A flow rate of 2-3 ml/minute is adequate.

The GFR may not be performed at flow rates less than 1 ml/minute.

2.Water loading serves to increase urine volume and the frequency of spontaneous voiding. The use of spontaneously voided urine should decrease the likelihood of incomplete bladder emptying, a source of possible error. Bladder emptying should be assessed using ultrasound in the screening period in patients with symptoms suggestive of lower urinary tract obstruction (frequency, hesitancy, diminished urinary stream). In these patients a pre- and post-void echo of the bladder will be obtained after an oral water load of 500 ml. If the patient develops these symptoms during Baseline, see Protocol, Exclusion Criteria.

3.Accurate timing of urine collections and careful measurement of urine samples are essential. The timing of the urine samples is critical. All times are recorded based on the time of completion of the urine collection and recorded to the nearest minute using an accurate timing device such as a digital clock. The voiding intervals will vary since they are based on spontaneous voidings. Subjects with lower GFR function may excrete the water load slowly.

4.All Biochemistry samples must be drawn prior to the Glofil injection.

5.The heparin saline solution used in drawing the GFR blood samples is made by drawing 1000 unit per ml sodium heparin into a 5 ml. syringe down to the 0.5 ml line and then diluting with 0.9% saline to a total of 5 ml. This solution must be well mixed by rolling the syringe vigorously between the palms of your hands. Never use 10,000 unit per ml. heparin. Always check the bottle prior to use.

6.A locally approved individual will inject the Glofil subcutaneously in the upper arm region. This will be provided in 1/2 cc. insulin syringes as a sterile, pyrogen free solution containing 35 micro curies per dose. The entire volume (approximately 0.2 cc) is injected subcutaneously at one site. A skin fold in the side of the upper arm will be grasped and the needle inserted at a 90· angle up to the needle hub. Refer to Section 7.11 for more information on ^{125}I-sodium iothalamate and radiation safety.

7.After the study, the patient should be encouraged to maintain a high urine output and to void frequently to minimize radiation exposure to the bladder.

8.The following conditions make the GFR sample inaccurate.

a.An incomplete urine collection makes the GFR period unacceptable. Collect another period.

b.When another isotope contaminates the patient samples and its interference cannot be subtracted or allowed to decay to background, the GFR cannot be calculated.

c.If fecal contamination occurs, collect an additional period.

d.If the flow rate is less than 1 cc/minute, the GFR is not accurate.

9.Refer to References at the end of this chapter.

7.3 Central Laboratory Responsibilities

7.3.1 Communication

Telephone inquiries having to do with aspects of GFR test performance, GFR data forms, etc. may be directed to the GFR Central Laboratory at the Cleveland Clinic Foundation: (216) 444-4552 or (216) 444-5040. Calls to these number will generally be answered on weekdays only, between 7:00 a.m. and 3:30 p.m. Eastern Time.

Written inquiries should be addressed to the GFR Central Lab at the address listed in the Address Directory or Section 7.2.2.

The GFR Central Lab will communicate sample receipt problems, protocol changes, and other information to the participating Clinic Center personnel as needed at the addresses/phone number listed in the most recent Study Address Directory.

7.3.2 GFR Sample Receipt

GFR samples will be sent by participating centers via commercial express delivery to the Central GFR Laboratory. The express service will be that service, specified by NIH, which holds the contract, and grants the lowest rates, for this study, and the GFR Lab will provide airbills/packing slips, account numbers, etc. as appropriate. Upon receipt, mailers will be logged-in and the contents will be inspected. No samples should be sent on Friday so that they would arrive on a weekend.

7.3.3 GFR Sample Counting

One-half ml aliquots of GFR samples will be pipetted into counting vials using a 500 microliter pipettor which is routinely checked (See GFR Laboratory Quality Control Section) for volumetric accuracy and precision. Samples will be identified by printing specific sample designations on the tube tops with permanent markers. The full set of GFR samples from each patient will be placed in counter racks in the following order (listed from first to last counted tube): background urine, U1, U2, U3, U4, background serum, S0, S1, S2, S3, S4. One ^{125}I standard will precede the first GFR tube counted in any run and a second like standard will follow the last tube counted. These standards will be made at the GFR Central Lab and will consist of actual ^{125}I-sodium iothalamate diluted as follows: 0.10 ml of undiluted ^{125}I-sodium iothalamate (as received from Isotex, Inc.; approximately 1.0 mCi in 4.0 ml) will be dispensed from a 0.5 ml insulin syringe into a Class A 500 ml volumetric flask and will be diluted to the line with water. After mixing by inversion 8-10 times, small aliquots of this standard solution will be retained in the GFR Lab; 500 microliter volumes of the solution will be pipetted using a calibrated pipettor into vials for the gamma counter. The two standards must match to within $\pm$5%. The standards will be used to detect gamma counter power supply changes or other problems occurring during counting of a series of tubes. The principal gamma counter for the study samples will be a Packard

5550 Gamma counter. The instrument has three counting channels. The channel A window will be set to count 15-80 keV, channel B will count 80-470 keV, and channel C is set to count 15-80 keV. Instruments will be calibrated versus a ^{137}Cesium standard to insure accurate window settings. Channels A and C should count ^{125}I identically (within dial accuracy and statistical variation), while channel B will detect any ^{131}I, ^{99}mTc, etc., which should be not be present in any case in this study. Counter parameters will be: standard deviation = 1.5%, maximum count time 10 min. and low count reject = 60 counts/15 sec. These parameters will ensure that samples of $\geq$ 450 counts per minute will be counted to 4,500 total counts/1.5% S.D. In the event that the Packard counter should fail, a second Packard 5550 Gamma Counter is available as an alternate counter.

7.3.4GFR Test Data Forms

The GFR Central Lab will receive the Form #24, GFR Procedures Form, with each set of patient GFR samples. The information from this form will be used to fill out the Central Lab GFR Report Form, and to provide test information for the GFR calculation program. The written forms will be inspected for completion and accuracy.

7.3.5GFR Calculations

GFR calculations will be done as described in Section 7.5. GFR calculations will be done using an in-house GFR program. This program will calculate the individual GFR periods, the average GFR, the GFR calculated as one period and the coefficient of variation. The coefficient of variation is defined as:

$$\frac{\text{Standard Deviation}}{\text{Average GFR}} \times 100\% \quad .$$

This program includes provisions for data/result storage and reporting.

7.3.6Reporting of GFR Results

The GFR reports will include the four individual GFR periods, the average GFR, the GFR calculated as one period, and the GFR coefficient of variation.

Central GFR personnel will review variable GFR's and discuss the tests with the responsible technologists. If the CV for G1 is over 50%, the G1 may be repeated one time. If the repeat G1 is then in range with CV under 50%, the patient would continue to G2. Otherwise the patient would be excluded. If the CV for G2 is over 50%, the G2 may be repeated one time unless the G1 had a CV over 50%. In this situation, the patient would be excluded. **There will not be a fourth Baseline GFR.** If the repeat G2 was in range and had a CV under 50%, the patient would be eligible for randomization. However, during follow-up, if a CV for a GFR is >50%, the GFR does not need to be repeated.

A complete GFR consists of four periods. Occasionally a three period GFR will be acceptable, but the Clinical Center must consult with the Central GFR Lab before discontinuing a test. No two period GFR results will be allowed.

GFR reports will be sent via local network to the Data Coordinating Center.

Because Clinical Center staff members are blind to GFR results, the Data Coordinating Center will notify the involved Clinical Center when a GFR Action Item occurs. The GFR must be repeated. If the repeat GFR is at an acceptable level, the patient may continue in the Study. If the repeat GFR is at an unacceptable level, the Data Coordinating Center will notify the Clinical Center that a GFR stop point was reached.

7.3.7GFR Data Storage

The GFR Worksheet (Form #24) and Central GFR Lab Report will be filed by the Data Coordinating Center. The GFR data will also be stored in the study data base and these files will be accessible for result reporting. The GFR Lab will file its copy of Form #24 with the gamma counter results and a copy of the GFR software output for each GFR processed.

7.3.8GFR Laboratory Radiation Safety

The GFR Central Lab is regularly monitored by the lab technologists themselves as well as by the CCF Radiation Safety Officer. Regular records of these inspections are filed in the Lab/Radiation Safety Office. In general, the lab countertop surfaces are regularly washed with Radiacwash or a comparable decontaminating detergent, and are covered with plastic-backed absorbent sheets to contain any radioactive sample losses for disposal. Such contaminated material is collected and bagged as contaminated solid trash. This trash is picked up by CCF housekeeping staff. The GFR Lab does have an approved disposal sink for water disposal of ^{125}I-sodium iothalamate; handling of study samples will probably not require any such disposal. GFR Lab personnel wear quarterly interval gamma radiation film badges and follow all CCF policies concerning the handling of radioactive materials. Standards will be stored appropriately. Counter background activity will be monitored and clean up of inappropriate levels initiated promptly.

7.3.9GFR Laboratory Quality Control

Internal quality control of the Central GFR Lab is accomplished as follows:

1.The pipettor used for GFR samples is evaluated for volumetric accuracy and precision at 6-month intervals using the weighing of water on an electronic microbalance as a quality control technique.

2.The gamma counter is calibrated with ^{135}Cesium standards to assure accurate peak locations/window settings.

3.The patient counts are bracketed by matched ^{125}I-sodium iothalamate standards to eliminate instrumental malfunctions during sample counting as an error source.

4.The counter efficiency is monitored daily using ^{137}Cesium standards. Counter background activity is monitored on a daily basis as well.

5.A precision study is run weekly by randomly selecting a GFR study and rerunning it.

See Section 10.7 of the Manual of Operations for GFR quality control instructions.

7.3.10 Certification of Clinic Center GFR Personnel

GFR Technician

At the beginning of the Study, GFR personnel from all Clinical Centers will receive Central Training in the performance of the GFR test. This instruction will include theory and practice of GFR testing in some detail, radiation safety, and a demonstration of the GFR protocol. This course will be given by the GFR Central Lab Staff at the Cleveland Clinic. Clinic Center AASK Technicians will be certified by this staff upon successful completion of twelve GFR's. If, after the study start-up, a new untrained technologist assumes the GFR responsibilities from the previously trained technologist, this person will be required to attend an abbreviated Central Training Session at the Central GFR Laboratory.

GFR Back-Up Technician

Each center must have a back-up technician trained by the Central GFR Lab. The back-up technician is required to perform six GFR's a year. These six GFR's should be spaced throughout the year, so the back-up will be competent to perform the duties of the technician when they are not available.

7.3.11 Procedure for Detecting Possible Radioisotope Misadministration

An upper level of radioactivity for serums has been established by the Central GFR Lab to detect possible overdoses. If a study serum count exceeds 2750 cpm, the GFR Technician involved in that study will be contacted. It will be their responsibility to investigate the incident for a possible overdose. If an overdose did occur the Principal Investigator of that center and the Chairman of the Quality Control Committee will be notified. It is the responsibility of the Clinical Center's Principal Investigator to fulfill the reporting requirements listed in the Procedure for Reporting Misadministration of Radioisotope during GFR studies (see Section 7.11).

7.3.12 Mailing Supplies for GFR Samples

The Central GFR Laboratory will provide all necessary supplies for the mailing of GFRs. This includes styrofoam insulated mailing containers with cardboard outer mailing boxes, 5 ml. polypropylene serum and urine mailing tubes, zip-lock type plastic bags, freezer packs, packing tape, and labels.

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 GFR Lab shortly after each set of patient samples is received. Styrofoam mailing containers and freezer packs will be re-used whenever possible and replaced by the Central GFR Lab. Plastic sample mailing tubes and zip-lock bags are discarded by the Central GFR Lab after each mailing and replaced. Supply Order Forms will be sent back to Clinical Centers with each re-mailing of shipping boxes (see Form #75). Clinical Center technologists may check off needed supplies on these forms and return them to the GFR Central Lab with the next sample mailing to indicate their need for additional supplies.

7.3.13 Shipment of Samples

Samples will arrive as next-day mail, and packages are delivered directly to the GFR Lab.

The Central GFR Lab will log-in samples received. In the event that mailing difficulties occur, the Clinical Center which sent the package should follow up as needed when notified of a problem. Participating Clinical Centers should keep a log of sampling mailing dates and tracking numbers for reference. The Central GFR Lab will track packages within the Cleveland Clinic Foundation.

7.4 References

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7.5 Details of GFR Calculations

Introduction:

GFR is equal to UV/P U = activity in urine, corrected for background
P = activity in serum, corrected for background
V = urine volume/time of collection period

The serum activity for any given period will be the log mean of the serum sample counts at the beginning and end of that period. For example, the serum activity corresponding to urine #1 is derived from BLOOD #0 and BLOOD #1. The logarithms used are to the base 10.

$$GFR = \frac{U_1 \times V_1}{10^{(\text{Log } B_0 + \text{Log } B_1)/2}}$$

All GFR measurements will be corrected to $1.73m^2$ using the most recent height measurement and the weight determined on the day of the study. The BSA can be obtained from the nomogram of Boothby and Sandiford; it is calculated at the Central GFR Lab. (See Figure 1.)

The GFR for a given study will be calculated two ways. First, the GFR for each of the four periods is calculated; these are then averaged (Figure 2). The coefficient of variation of the four results is calculated using this average as an indication of the variability of the GFR. In addition, the GFR will also be calculated as if there were only one long collection period (Figure 3). This will be performed using time-averaged serum counts and volume-averaged urine counts. This GFR is the actual outcome value which is stored in the database as the patient GFR.

Figure 1

The program used at the Central GFR Laboratory calculates body surface area according to the following formula:

$$BSA \text{ (in } M^2) = [W^{0.425} \times H^{0.725} \times 71.84]/10{,}000$$

where W = weight in kilos
H = height in centimeters

Figure 2

Sex (0=M, 1=F): 1
Weight (kg): 55.1 Height (cm): 155

Time Period	Actual Time Hrs		Min	Elapsed Time (min)	Urine Volume	Serum Count	Urine Count	GFR
B	09	:	20			^{B}Back = 20	^{U}Back = 25	
0	10	:	21	61		^{B}0 = 2319		
1	11	:	04	43	94.0	^{B}1 = 2121	^{U}1 = 14443	16
2	11	:	47	43	86.0	^{B}2 = 1801	^{U}2 = 11967	14
3	12	:	31	44	96.0	^{B}3 = 1695	^{U}3 = 10830	15
4	13	:	12	41	86.0	^{B}4 = 1516	^{U}4 = 9847	15

Average GFR: 15.1 GFR as 1 period: 15 CV:6

Body Surface Area (BSA) = 1.53m^2

Urine Flow Rates (V)
Period 1: 94.0/43 = 2.19 ml/min

Period 2: 86.0/43 = 2.00 ml/min

Period 3: 96.0/44 = 2.18 ml/min

Period 4: 86.0/41 = 2.10 ml/min

Log Mean Serum Counts (P)
$P_1 = 10^{(\text{Log}(2299) + \text{Log}(2101))/2} = 2198$

$P_2 = 10^{(\text{Log}(2101) + \text{Log}(1781))/2} = 1934$

$P_3 = 10^{(\text{Log}(1781) + \text{Log}(1675))/2} = 1727$

$P_4 = 10^{(\text{Log}(1675) + \text{Log}(1496))/2} = 1583$

GFR Corrected for BSA ($^{UV}/_{P}$)

P_1: $\frac{14418 \times 2.19}{2198} \times \frac{1.73}{1.53} = 16.25$ ml/min/1.73m^2

P_2: $\frac{11942 \times 2.00}{1934} \times \frac{1.73}{1.53} = 13.96$ ml/min/1.73m^2

P_2: $\frac{10805 \times 2.18}{} \times \frac{1.73}{} = 15.42$ ml/min/1.73m^2

Avg. GFR = 15.09

SD = 0.98

CV = $\frac{0.98}{}$ x 100% = 64%

$$P_4: \quad \frac{\frac{1727}{9822 \times 2.10}}{1583} \times \frac{1.53}{1.73}{1.53} = 14.73 \text{ ml/min/1.73m}^2 \qquad 15.09$$

Average GFR Calculation For a Four-Period GFR

See the data columns listed in Figure 2 on the previous page. Serum and urine counts for ^{125}I are labeled with the sample designations currently used. First, we calculate the body surface area from patient's height and weight values using the following formula:

$$BSA \text{ (in } M^2) = [W^{0.425} \times H^{0.725} \times 71.84]/10{,}000$$

where W = weight in kilos
H = height in centimeters

In this example, BSA = $1.53m^2$ to two decimal places.

To determine GFR calculations, first subtract the background serum count from each of the subsequent serum counts; subtract the background urine count from each of the subsequent urine counts similarly. Next, calculate the urine flow rates for the four urine collection periods; for example, the flow rate for Period 1 is 94.0 ml divided by 43 minutes, which gives 2.19 ml/min. Next, calculate the log mean serum counts for each collection period. For period one, the B_0 blood sample was drawn when the discard urine was collected and the blood sample B_1 was drawn when the urine U_1 was collected. Therefore, the samples B_0 and B_1 bracket the U_1 collection period and their log mean value is defined as:

$$10^{[(\text{Log}(B_0 - B_{Background}) + \text{Log}(B_1 - B_{Background}))/2]}$$

Filling in the counts, this value becomes ten raised to the (Log 2299 + Log 2101)/2 power, which is $10^{3.341982512} = 2197.77 = 2198$. The log mean values for the remaining periods use their respective bracketing blood sample counts.

Finally, the UV/P calculation is done, where U represents the urine count for a period minus the urine background, V represents the urine flow rate for that period, and P is the log mean serum count for the same period. For period one, U is U_1 counts minus $U_{Background}$ counts = 14418, and GFR = (14418 x 2.19)/2198 = 14.37 ml/min. This is then corrected for body surface area by multiplying 1.73/BSA; GFR = (14.37)(1.73/1.53) = 16.25 ml/min/$1.73m^2$. Other period GFR values calculated in like fashion. The average of these individual period GFR values then stored as the average GFR. The average GFR is used only to calculate coefficient of variation (CV). The calculation program also calculates the standard deviation (SD) of the four GFR values and reports the coefficient of variations data, where CV is defined as CV = (SD/average GFR) x 100%.

Figure 3

Sex (0=M, 1=F): 1
Weight (kg): 55.1 Height (cm): 155
Average Creatinine Clearance: Average Urea Clearance:

Time Period	Actual Time Hrs	Actual Time Min	Elapsed Time (min)	Urine Volume	Serum Count	Urine Count	GFR
B	09	20			^{B}Back = 20	^{U}Back = 25	
0	10	21	61		^{B}0 = 2319		
1	11	04	43	94.0	^{B}1 = 2121	^{U}1 = 14443	16
2	11	47	43	86.0	^{B}2 = 1801	^{U}2 = 11967	14
3	12	31	44	96.0	^{B}3 = 1695	^{U}3 = 10830	15
4	13	12	41	86.0	^{B}4 = 1516	^{U}4 = 9847	15

Average GFR: 15.1 GFR as 1 period: 15 CV:6

Body Surface Area (BSA) = 1.53 m^2

Total Urine Volume = 362 ml
Total Test Time = 171 min
(Periods 1-4)

Log Mean Serum Counts (P)
Period 1: 2198
Period 2: 1934
Period 3: 1727
Period 4: 1583

Urine Counts Minus Background (U)
Period 1: 14418
Period 2: 11942
Period 3: 10805
Period 4: 9822

Urine Counts Weighted by Volume

$$U = \frac{14418\ (94)}{362} + \frac{11942\ (86)}{362} + \frac{10805\ (96)}{362} + \frac{9822\ (86)}{362} = 11780$$

Log Mean Serum Counts Weighted by Time

$$P = \frac{2198\ (43)}{171} + \frac{1934\ (43)}{171} + \frac{1727\ (44)}{171} + \frac{1583\ (41)}{171} = 1863$$

GFR As One Period

$$\frac{\frac{11780 \times (362/171)}{1863}}{1.53} \times 1.73 = 15.1 \text{ ml/min/1.73m}^2$$

GFR Calculation As One Long Collection -- For a Four-Period GFR

Calculations are identical with those shown in Figure 2 for the average GFR calculation through the log mean P values. Then, as shown in Figure 3, the four P values are weighted by their respective period time/total time ratios and combined. Likewise, the urine counts U for each period are weighted by their respective period volume/total volume ratios and combined. The GFR value is then determined by a single calculation employing these combined U and P values and the total urine volume divided by the total test time. BSA correction is as usual. This "One Long Collection" weighted GFR is the GFR measurement used as the official AASK Protocol defined GFR for entry and slope calculations.

7.6 ^{125}I-Sodium Iothalamate (Glofil)

The sole supplier of Glofil in the United States at the present time is Cypros Pharmaceutical Corporation, 2732 Loker Avenue West, Carlsbad California 92008, phone 1- 800-411-3065. The material is synthesized on a monthly schedule and is available in 4 ml aliquots (1.0 milli Curies). It is stored at 4·C in its lead container in a suitable nuclear medicine area and is stable for 45 days according to the manufacturer, although the half-life of ^{125}iodine is 60 days. The limit to Glofil's usable life is a function of the chemical instability of the isotopic label, which is slowly released as free ^{125}iodine from the iothalamate molecule. As this free ^{125}iodine accumulates, the clearance of Glofil deviates from the true GFR. The material should not be used past the manufacturer's expiration date.

The iothalamate is drawn up in a 1.0 or 0.5 ml plastic insulin syringe with a 28 gauge x 1/2 inch needle. The total activity in the dose for an adult patient should be 35 micro Curies. The syringe weight/activity before and after the shot is not measured, and no standard solution of the iothalamate is required. The iodine-allergic patient should not be given the iothalamate.

AASK Technicians and other Clinical Center personnel handling the iothalamate should take all safety precautions to protect both the patient and themselves from unnecessary radiation exposure. Technologists should wear appropriate gamma ray-sensitive film badges and follow their exposure levels; the iothalamate should be stored and delivered in shielded containers. ^{125}iodine emits low energy gamma radiation with a maximum energy of 36 keV and will shield the user very effectively (1 mm of lead will stop 99.96% of the radiation from an ^{125}I source). The syringes may be handled briefly, while the shot is given to the patient, without shielding. Gloves should be worn in case dose leakage or other unsafe conditions arise. All syringes, needles, empty isotope bottles and any other radioactive trash should be disposed of via established contaminated refuse protocols (consult radiation safety and/or disposal regulations); as a rule of thumb, any isotope decays to less than 1% of its original activity in seven half-lives; this is 420 days for ^{125}iodine. Small amounts of ^{125}iodine may be disposed by flushing with large amounts of water down the drain in approved disposal sinks.

7.7 Dose Estimates for Skin and Other Organs

Dose Estimates for the Skin

The dose estimates to the skin from subcutaneous injection Glofil are shown in the table below. These estimates are based on the following assumptions; injection of 35 Ci in a volume of 0.2 ml, 3 minute effective half time at the injection site, site modeled as a disk of radium 1 cm and thickness 0.064 cm, disk directly beneath the skin surface and in contact with skin. The distances, then, are the distances from the face of the disk into the overlying skin.

	Dose (rad/35 Ci)		
Distance (cm)	Photon	Beta	Total
0.0015	0.05	0.0035	0.0535
0.002	0.0475	0.000425	0.048
0.003	0.045	----	0.045
0.008	0.0375	----	0.0375
0.018	0.030	----	0.030
0.028	0.0275	----	0.0275
0.038	0.0248	----	0.0248

Dose Estimates for Major Body Organs

The dose estimates for major body organs for intravenous administration of Glofil are shown in the table on the following page. These estimates may be used interchangeably as estimates for subcutaneous injection due to the long physical half life of ^{125}I. These estimates are based on essentially the same distribution data as the estimates in the Abbott package insert (done in 1972), but using improved calculational techniques and including the dose to the urinary bladder, which turns out to be very important. No thyroid dose has been calculated.

Radiation Dose Estimates for Skin and Other Organs*

	Estimated Radiation Dose			
	2.0 hours **		4.8 Hours**	
	mGy	rad	mGy	rad
Organ	MBq	mCi	MBq	mCi
Bladder	0.058	0.21	0.16	0.058
Kidneys	0.016	0.059	0.016	0.059
Liver	0.0040	0.015	0.0040	0.015
Ovaries	0.0015	0.0055	0.0026	0.0095
Red Marrow	0.0015	0.0057	0.0017	0.0063
Testes	0.0051	0.019	0.0057	0.021
Total Body	0.0014	0.0053	0.0021	0.0077

*Assumed distribution and retention

Liver	11.52%	tb = 1.72 hr
Kidneys	10.26%	tb = 1.72 hr
Testes	0.41%	tb = 1.72 hr
Remainder	77.81%	tb = 1.72 hr

**Bladder voiding interval

Acknowledgment

The data regarding dose estimates for the skin were provided by Michael Stabin, Radiopharmaceutical Internal Dose Information Center, Oak Ridge Associated Universities (ORAU) in April, 1985.

The address for ORAU is P.O. Box 117, Oak Ridge, Tennessee 37831-0117.

7.8 Pre-GFR Light Meal Ideas

Before you consider use of a pre-GFR light meal, discuss this with the Cental GFR Lab staff. You may choose one of the sample menus below or you may choose your own combination of foods from the list following the menus. The meal should contain less than two (2) grams of protein and it should be eaten two (2) hours or more before the GFR begins. Note: DO NOT drink caffeinated beverages during the four (4) hours before the GFR procedure begins.

Sample Menus for Pre-GFR Light Meals

Serving Size (grams)		Protein
8 fl. oz.	Cranberry Juice	0.0
1	Rice Cake	1.0
2 Tbsp.	Apple Butter	0.2
1/2 cup	Peach Halves, canned, heavy syrup	0.6
	Total:	**1.8**
8 fl. oz.	Diet Soda	0.0
1 bar	Quaker Chewy Granola Bar	1.0
1 fruit	Apple (2-3/4" diameter)	0.3
Total: 1.3		
8 fl. oz.	Apple Juice	0.3
4 crackers	Saltines (1-7/8" square)	1.2
1 tsp.	Margarine	0.0
1 tsp.	Jelly	0.0
	Total:	**1.5**
4 fl. oz.	Grapefruit Juice	0.7
1/2 cup	Puffed Rice	0.5
2 fl. oz.	Nondairy Liquid Creamer	0.4
3	Low Protein Vanilla Wafers	0.1
	Total:	**1.7**
8 fl. oz.	Ginger Ale	0.0
2 slices	Low Protein Rusks, Aproten	0.2
2 Tbsp.	Jelly	0.0
1/4 cup	Raisins	1.2
1/2 cup	Applesauce, canned (sweetened or unsweetened)	0.2
	Total:	**1.6**
4 fl. oz.	Imitation Dairy Drink, Alterna	1.2
2	Low Protein Spice Cookies	0.2
1/2 cup	Fruit Cocktail	0.5

Total: 1.9

7.9 Foods to Choose From for the Pre-GFR Light Meal

Before you consider use of a pre-GFR Light Meal, discuss this with the GFR Lab staff. For your pre-GFR light meal, you may choose from the following list any combination of foods that total two (2) grams of protein or less.

Snacks	Amount	Protein (gm)
Quaker Chewy Granola Bars		
Apple or Strawberry	1 oz. bar	1.0
Rice Cake, all flavors	1 cake	1.0
Melba Toast, all flavors	1 round	0.4
Low Protein Rusks, Aproten*	1 slice	0.1
Low Protein Gelled Dessert*	1/3cup	0.1
Low Protein Cookies*		
Vanilla/Chocolate Wafers	3 wafers	0.1
Spice Cookies, Med Diet	1 cookie	0.1
Cereals*		
Sugar Frosted Flakes	1/2 cup	0.9
Rice Krispies	1/2 cup	0.9
Kix	1/2 cup	0.9
Puffed Rice	1 cup	0.9
Canned Fruit*		
Peach Halves, heavy syrup	1/2 cup	0.6
Fruit Cocktail, light syrup	1/2cup	0.5
Pear Halves, heavy syrup	1/2 cup	0.2
Pineapple, light syrup	1/2 cup	0.5
Applesauce, sweetened	1/2 cup	0.2
Fresh Fruit		
Apple	1 apple (2-3/4" diameter)	0.3
Orange	1 medium (2-5/8" diameter)	1.2
Pineapple	1 cup diced pieces	0.6
Banana	1 medium (8-3/4" long)	1.2
Grapes	10 grapes	0.3
Dried Fruit*		
Raisins	1/4 cup	1.2
Apricot Halves	4 halves	0.5
Dates	5 dates	0.8
Toppers		
Apple Butter	1 tablespoon	0.1
Jelly	1 tablespoon	0.0
Margarine	1 teaspoon	0.0

Juices and other Drinks*		
Apple Juice	8 fl. oz.	0.3
Grapefruit Juice	4 fl. oz.	0.7
Pear Nectar	8 fl. oz.	0.2
Cranberry Juice Cocktail	8 fl. oz.	0.0
Orange Drink	8 fl. oz.	0.0
Ginger Ale	8 fl. oz.	0.0
Miscellaneous Items		
Nondairy Liquid Creamer	4 fl. oz.	0.8

*****These foods can also be found in the grocery store in single-serving sizes. If you decide to purchase these snack pack items, please be sure to check the weight and the protein value listed on the label.

7.10 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.

7.10.1 Quality Control

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.

7.10.2 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.

7.11 Procedure For Reporting Misadministration of Radioisotope During GFR Studies

It is the responsibility of the Clinical Center Principal Investigator to fulfill the reporting requirements listed below in the event of a radioisotope misadministration during GFR procedure:

1. An error in isotope administration may be identified by clinical center personnel or by staff of the Central GFR Laboratory. Errors which result in misadministration of more than the prescribed dose must be reported immediately to the Quality Control Committee Chairman. In the event that the error is detected by the staff of the GFR Central Lab, it will be reported directly to the Clinical Center Principal Investigator and GFR Technician and to the Quality Control Committee Chairman by the GFR Lab Director.
2. The local institution radiation safety officer must be consulted to determine if the error is classified a "misadministration" according to the Nuclear Regulatory Commission, or for "agreement states", according to the state regulations. The Quality Control Committee must be notified, in writing, of the determination. If a misadministration has occurred, it must be investigated and reported in compliance with federal or state law, and copies of all subsequent correspondence and reports should be forwarded to the Quality Control Committee for review. If a misadministration has not occurred, no further investigation or reports are necessary, unless requested specifically by the radiation safety officer, the Quality Control Committee or NIH.
3. In the event of a misadministration, an investigation by Clinical Center Staff and the institution's radiation safety officer will be requested to provide the following information to the Quality Control Committee within three months of the occurrence or notification of the misadministration (unless stipulated otherwise by federal or state regulations):

a. The date of each misadministration and the study subject involved.
b. The amount of radioisotope administered and an estimation of the radiation absorbed dose for each misadministration.
c. A statement from the radiation safety officer whether the patients' safety was compromised; i.e., whether the radiation dose administered or absorbed exceeds the acceptable limits for study subjects and whether performance of subsequent GFR measurement is permissible.
d. A statement from the investigational review board whether the patient's consent was compromised; i.e. whether the dose administered or absorbed exceeds the amount stated on the consent form and whether performance of subsequent GFR measurements is permissible under the original consent.
e. A statement from the radiation safety officer whether it is necessary to notify the patient and the referring physician.
f. A statement from the radiation safety officer how the misadministration occurred and what steps have been taken to correct the circumstances which led to the misadministration.

4. The Quality Control Committee will review the above reports and will provide NIH with interim and final reports regarding the manner in which the misadministration is addressed and resolved.