# Modification of Diet in Renal Disease Study

## Manual of Operations
## Volume 1, Chapter 3
## The Clinical Center MDRD Technicians' Section

October 1988

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MODIFICATION OF DIET IN RENAL DISEASE STUDY

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CHAPTER 3 TABLE OF CONTENTS*



* Each part has its own detailed Table of Contents.

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## CHAPTER 3, PART 1

### Introduction and Job Description

Each of the Clinical Centers participating in the Modification of Diet in Renal Disease Study has an MDRD technician. This technician's primary responsibility is one of great importance to the study: the measurement of Glomerular Filtration Rate, or GFR. Each participating patient's GFR will be measured periodically over the course of the study, and the primary outcome variable upon which the results of the study will be based is the rate of change in GFR. The MDRD technician is also responsible for measuring blood pressure, processing laboratory samples and working on quality control.

The MDRD technician should be thoroughly familiar with the study Protocol, with this Chapter of Volume I of the Manual of Operations, and with relevant forms from Volume II of the Manual of Operations. The Protocol gives an overall picture of the MDRD study. The forms are used to collect the study data. This Chapter gives detailed descriptions of tasks of the MDRD technician related to measuring GFR, handling lab samples, collecting lab specimens, and assisting in quality control.

# Chapter 3, Part 2
# GFR Activities

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## 2.1 Laboratory Responsibilities

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

B. The Clinical Centers will be responsible for performing the GFR test and shipping the processed samples to the Central GFR Lab, as well as completing the GFR data form (Form #16).

## 2.2 Telephone/Written Communications

A. Telephone inquiries having to do with aspects of GFR test performance, GFR data forms, etc., may be directed to the Central GFR Laboratory at The Cleveland Clinic Foundation: (216) 444-4552 or (216) 444-5040. Calls to this number will generally be answered between 9 a.m. and 5:00 p.m., Eastern Time, weekdays only.

B. Written inquiries should be addressed to the GFR Central Lab at the address listed in the MDRD Address Directory or by electronic mail.

C. The GFR Central Lab will communicate with participating Clinical Centers, if need be, at the addresses/phone number listed in the most recent MDRD Study Address Directory.

## 2.3 GFR functions at the Central Lab

### 2.3.1 GFR Processing and Calculating

GFR samples will be sent via next day mail to the Central GFR lab. Upon receipt the samples will be logged in. Samples will be pipetted into counting vials, and 0.5 ml of sample will be counted on a Packard 5550 Gamma counter for two minutes each. GFR calculations will be done on the CCF VAX system using an in-house GFR program. The program will calculate the individual GFR periods, the GFR as one period, and the coefficient of variation. The coefficient of variation is defined as:

$$CV = \frac{\text{Standard Deviation}}{\text{Average GFR}} \times 100\%$$

The program includes provisions for data storage and reporting. See Appendix I at the end of this chapter for a detailed explanation of GFR calculations.

2.3.2 Reporting of GFR Results

The GFR results will be reported as follows:

a. During baseline, the GFR reports will include the four individual GFR periods, the GFR calculated as one period, the GFR coefficient of variation, the urine flow rates for each period, and the urine flow coefficient of variation.

b. During follow-up, the GFR coefficient of variation will be reported, the urine flow rates, and the urine flow CV.

Central GFR personnel will review variable GFR's and discuss the tests with the responsible technologists. If the CV of the GFR at BV3 exceeds 35%, this value cannot be used for randomization. The GFR may be repeated once within one month. If the repeat GFR has a CV greater than 35%, the patient is excluded from the study. Given that the GFR Worksheet Form #16 has been transmitted to the DCC, the GFR reports will be sent out by electronic mail.

A MDRD GFR consists of four periods. Occassionally a three period GFR will be acceptable, but the Clinical Center must consult with the Central GFR Lab before discontinuing a test. A two period GFR will be allowed at the B0 visit, if an attempt was made to do four periods.

2.3.3 GFR Data/Result Storage

The GFR Worksheet (Form #16) 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 via electronic mail and/or printed mail as needed. The GFR Lab will file its copy of the Form #16 with the gamma counter results and a copy of the GFR software output for each GFR test processed.

2.3.4 Certification of Clinical Center GFR Personnel

At the beginning of the MDRD Study, GFR personnel from all Clinical Centers will receive Central Training in the performance of GFR

tests. This instruction will include theory and practice of GFR testing in some detail, radiation safety, and a GFR employing subcutaneous injection of $^{125}$I-sodium Iothalamate (Glofil) will be performed during the instruction period. This course will be given by the GFR Central Lab Staff at the Cleveland Clinic.

Clinical Center MDRD 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. 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.

## 2.4. Clinical Centers' GFR Activities

### 2.4.1 GFR Test Protocol

The certified 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 MDRD 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:

#### 2.4.1.1 Principle

Urinary clearance of Glofil 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 iodide (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.

2.4.1.2 Materials and Equipment

1. To perform GFR
   a. Saturated solution of potassium iodide (SSKI)
   b. Scale to weigh patient
      (The person should be weighed as part of the monthly physical exam, and only a properly calibrated scale should be used. See the Dietitians' Chapter 1 for scale calibration requirements.)
   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).

2.4.1.3 Procedure

1. Check eligibility. The patient has been fasting for 12 hours. The patient cannot have anything by mouth except

water for 12 hours or more prior to the test. The MDRD technician should certify this with the patient to uncover the patient who forgot to fast. Non-steroidal anti-inflammatory agents, including aspirin, cimetidine, ranitidine, trimethoprim/sulfa, and trimethoprim will be withheld for at least 48 hours prior to each GFR test. Other drugs may be taken up to and including the morning of the GFR test, but the Keto Acid supplements are considered a food and should not be taken before a GFR.

Women of child bearing 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 cancelled if the test was positive or if the patient did not have the test. If the pregnancy test is done by MDRD personnel and the MDRD personnel find a positive test result, the MDRD personnel should not startle the patient by telling her that she is pregnant. It is preferred that the MDRD personnel send the patient back to her referring physician for interpretation of the pregnancy test results and possible confirmatory testing. Whether the pregnancy test is do outside the Clinical Center or at the Clinical Center, written resul must be on file or an Abbott Testpack HCG-COMBO Serum Pregnancy Test on the morning of the GFR. Complete instructions for the Abbott pregnancy test can be found in the Appendix to this chapter. If the patient has had a radionuclide diagnostic test in the past month, using an isotope other than $^{99}$Technetium, the GFR must be reschedule The GFR tech should verify that procedures have been followed for every test. (Refer to GFR Checklist in Appendix IV.)

2. The patient should have had a 5 ml/kg water load at home.
3. Mix 5 drops of SSKI in 20 ml of water and give to the patient orally.

   Note: time given. The SSKI prevents thyroid uptake of any free $^{125}$I; this protects the patient and elimates error in the GFR determination due to the additional elimination route for the isotope. (Any patient with a true iodine allergy is excluded from the MDRD Study).

4. Start hydrating the patient. A water load of 10 ml/kg should be given during the next 90 minutes. See Section 2.4.1.6 procedure notes items 1 and 2.
5. Collect a background urine; record the time. Measure the volume.
6. 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 a butterfly infusion set with a heparin lock. A recommended set is a 21 gauge x 3/4 inch infusion butterfly needle with a 12-inch tubing (Abbott #4492 or Argyle Division of Sherwood Medical #8888-111724) and Vacutainer tube holder with a screw-on luerlock adapter (Becton-Dickinson #7290). Use of an infusion set needle eliminates the common occurrrence of a hand held vacutainer needle being pulled out of or pushed through a vein during multiple tube changes. The 12-inch line allows free motion for tube changes with no needle shifts. An armboard should be used whenever the butterfly position will be jeopardized by a patient's arm motion.
   c. Insert the butterfly needle 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 Section 2.4.1.6 Procedure Notes, item 5.
   d. After heparinization, the line may be re-capped. The needle should be taped down securely with paper tape or some other easily removed hypoaller-genic tape. Avoid taping the needle down to an extreme degree; this may pinch off the flow of the blood.

7. At least 30 minutes following the administration of the SSKI, the Glofil is injected subcutaneously in the upper arm. See Section 2.4.1.6, Procedure notes, item 6 Record the time.
8. Continue to hydrate the patient at the rate of 200-400 ml/hour as tolerated throughout the study.
9. At least 60 min. after the time of the Glofil injection, the patient should void. Record the time (time #0). Measure the urine volume. See section 2.4.1.6, Procedure Notes, item 3. Determine the difference in time between the collection of the background urine sample and time #0. Divide the volume of the urine by this difference in times. If this flow rate value is at least 3 ml/min., then continue with the test. If it is less than 3 ml/min., wait a full 90 min. 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 continue with the test (See Form 16W).
10. Draw a blood sample (S-#0) using the heparin lock. To draw GFR samples, first clean the heparin from the line plus one ml. of blood to avoid diluting the GFR sample. Discard this initial diluted sample; then draw the GFR sample. 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.
11. In 30 min. 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 seems low, extend the period to get additional urine and a higher flow rate, as described in #9 above.)
12. Draw the next blood sample (S-#1). Reheparinize the line.
13. Repeat steps 11 and 12 until four timed urines have been collected and appropriate blood samples drawn.
14. When all the blood samples have been obtained, remove the needle. Have the patient apply moderate pressure at the site for five minutes to avoid bleeding. Then apply a bandage.

15. When the blood samples have clotted, prepare the samples for mailing to the Central GFR Lab as follows:
    a. Label the mailing tubes with the GFR labels. Be sure to include the patient's name code on each tube.
    b. Centrifuge the blood samples.
    c. Place an aliquot of serum in an appropriately labeled tube; save a backup sample in the refrigerator. (Discard the duplicate when GFR results are received unless you are asked to submit the backup sample labelled with the QCID for quality control of the Central GFR Lab.)
    d. Place an aliquot of urine in the appropriately labeled tube; save a duplicate sample in the refrigerator. (Discard the duplicate when GFR results are received.)
    e. Tighten all the caps of the mailing tubes.
    f. Prepare the mailer for shipping with frozen ice packs.
    g. Place all GFR tubes in a zip lock bag; place all biochemistry samples in a separate ziplock bag.
    h. Check appropriate GFR and Biochemistry forms to make sure that they are filled in completely.

    Refer to sample mailing instructions in section 3.4.11 of this chapter for information on packing.

2.4.1.4 OPTIONAL GFR DATA CHART:

PATIENT NAME CODE: _____ PATIENT ID NUMBER: _____

DATE: _____ VISIT NUMBER: _____ PATIENT'S WEIGHT: _____

| | ACTUAL TIME | WATER LOAD (ML) | URINE* | URINE VOLUME | FLOW RATE | BLOOD SAMPLE* |
|---|---|---|---|---|---|---|
| IODINE 5 drops in water | | | | | | |
| BACKGROUND SAMPLES | | | U-Bkgrd | | | S-Bkgrd |
| INJECTION Iothalamate I125 | | | | | | |
| DISCARD SAMPLE U-#0 | | | | | | S-#0 |
| PERIOD #1 | | | U-#1 | | | S-#1 |
| PERIOD #2 | | | U-#2 | | | S-#2 |
| PERIOD #3 | | | U-#3 | | | S-#3 |
| PERIOD #4 | | | U-#4 | | | S-#4 |

*Indicate with a check mark when collected or drawn.

2.4.1.5 GFR CHECKLIST (See Form #16, also)

1. Make sure that your patient is fasting, has not taken any restricted medications (see page 1.3.8), has not had any radionuclide diagnostic test recently, and has had a negative pregnacy test if necessary.
2. Make sure the patient does not have a short term intercurrent illness.
3. Give patient iodine; note the time.
4. Start hydration of 10 ml/kg over the next 90 minutes.
5. Collect the background urine; measure its volume. (U-B)
6. Obtain the background blood sample and any appropriate Biochemistry samples. (S-B)
7. At least 30 minutes after the iodine was administered, give the Glofil injection; note time.
8. 60-90 minutes later, have the patient void; note the time (Time #0); measure the urine volume.
9. Draw blood sample (S-0).
10. Continue hydrating patient at a rate of 200-400 ml/hr.
11. In 30 minutes or more collect next urine sample (U-1); note the time; measure the volume.
12. Draw blood (S-1).
13. Repeat steps 10-12 until four urine collections have been collected and the appropriate blood samples have been obtained.
14. When blood samples have clotted, prepare samples for mailing.

2.4.1.6 Procedure Notes

1. A well-hydrated patient is crucial to a valid GFR study. While 200-400ml/hour is the recommended water-load, the technicians should use their own good judgement based on the patient's kidney function to determine the appropriate amount of hydration. A desirable flow rate is 4-6 ml/min, 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/min.
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 upperarm region. This will be provided in 1/2c. Insulin syringes as a sterile, pyrogen free solution containing 35 microcuries per dose. The entire volume (approximately 0.2 cc) is injected subcutaneously at one site. A skin fold in the back of the upper arm will be grasped and the needle inserted at a 90 degree angle up to the needle hub. Refer to Appendix II at the end of this chapter for more information on 125I-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.

      The GFR technician should call the Central GFR Lab if he or she has any questions about any GFR.
   d. If the flow rate is less than 1 cc/min, the test is not accurate.
9. Refer to Appendix IV at the end of this chapter for references.

2.4.2 GFR Test Worksheet Completion (see MDRD Form # 16)

The GFR Technicians will complete the GFR Worksheet Form and include this with each GFR sample mailing.

2.4.3 GFR Test Troubleshooting

In cases with unusually discrepant results for the four periods, the GFR Central Lab will 'troubleshoot' to determine what factors might have caused the imprecise test.

2.4.4 Actions Based Upon GFR Test Results

GFR stop points are listed below (from the MDRD protocol section 10.3.3):

a. For Study A patients, a decline in GFR from Baseline Visit 3 (B3) to the level indicated below:

| B3 GFR | Follow-Up GFR |
|---|---|
| > 40 ml/min/1.73m2 | ≤ 20 ml/min/1.73m2 |
| 25 - 40 ml/min/1.73m2 | < 50% of B3 value |

Because Clinical Center staff members are blind to GFR results during Follow-Up, the Data Coordinating Center will notify the involved Clinical Center when a GFR Action Item occurs. The GFR must be repeated within one month. If the repeat GFR is above the level indicated above, then the patient continues in Follow-Up and the GFR is measured at the next routinely indicated visit. If the repeat GFR is at or below the level indicated above, the Data Coordinating Center will notify the Clinical Center that a GFR stop point was reached.

b. Study B and C patients. No GFR stop point exists; initiation of dialysis or transplantation is a stop point.

2.4.5 Procedure for detecting a possible radioisotope misadministration.

An upper level of radioactivity for serums was determined by the Central GFR Lab to detect possible overdoses. If a serum count exceeds this upper level the MDRD Technician involved in that study will be contacted. It will be his or her 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 Clinical Management 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 (Appendix IV).

# Chapter 3, Part 3
## Clinical Center Sample Collection and Handling

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## 3.1 Laboratory Responsibilities

A. The Central GFR Laboratory at the Cleveland Clinic Foundation will receive specimens for GFR only.

B. The Central Amino Acid Laboratory will receive and process Amino Acid Samples and report results.

C. The Central Biochemistry Laboratory at the Cleveland Clinic Foundation will receive and perform all Biochemistry testing and reporting of results.

D. The Clinical Center MDRD Technician will be responsible for collecting all samples, processing them, and mailing them to the appropriate Central Lab.

## 3.2 Telephone/Written Communications

A. Telephone inquiries having to do with aspects of sample collection/processing/mailing may be directed to the Central GFR Laboratory, the Central Biochemistry Laboratory, or the Central Amino Acid Laboratory. Calls to the GFR Lab will be answered between 7 a.m. and 5:30 p.m., Eastern Time, weekdays only. Call to the Central Biochemistry Laboratory will be answered between 8 a.m. and 4:15 p.m. EST. weekdays only. Calls to the Central Amino Acid Laboratory will be answered between 8 a.m. and 5 p.m., Central Time, weekdays only.

B. Written inquiries can be sent to the Central GFR Laboratory or the Central Biochemistry Laboratory, or the Central Amino Acid Laboratory at the addresses in the MDRD Address Directory, or by electronic mail.

C. The Central GFR Lab, the Central Biochemistry Lab and the Central Amino Acid Laboratory will communicate with participating Clinical Centers over electronic mail, or at the addresses/phone numbers listed in the MDRD Address Directory.

## 3.3. Central GFR and Biochemistry Laboratory

### 3.3.1 Procurement of Mailing Supplies for GFR Samples

The Central GFR Laboratory will provide all necessary mailing supplies for mailing of GFRs only. 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 suitable labels.

3.3.2 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 and 7 ml polypropylene serum/plasma mailing tubes, zip-lock plastic bags, freezer packs, packing tape, and suitable mailing labels. See Appendix XIV.

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

3.3.3. 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 GFR Lab, or Central Biochemistry 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, or CBL as needed. Plastic sample mailing tubes and zip-lock bags are discarded by the Central GFR Lab and Central Biochemistry Lab after each mailing and replaced. Supply Order Forms will be sent back to Clinical Centers with each re-mailing of shipping boxes (See Appendix III). Clinical Center technologists may check off needed supplies on these forms and return them to the GFR Central Lab or Central Biochemistry Lab with the next sample mailing to indicate their need for additional supplies.

3.3.4 Postal Inquiries

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

### 3.3.5 Sample Package Receipt at CCF

Samples will arrive as next-day-mail at CCF. Packages are delivered directly to the GFR Lab, or to the Central Biochemistry Lab.

### 3.3.6 Communication with Participating Clinical Centers Concerning Sample Receipt Problems, Protocol Changes, etc.

The Central GFR Lab or the Central Biochemistry Lab will communicate sample receipt problems, protocol changes, and other information to the participating Clinical Center personnel as needed.

## 3.4 Sample Handling at the Clinical Centers

### 3.4.1 Sample Requirements

The "Sample Requirements" table in Appendix VII lists the total volume of serum in mls needed to complete the various biochemical assays. The "Sample Requirements" table for action items can be found in Appendix XVI. The table has the number of tubes required to get the needed serum or plasma for CBL tests for each visit, for a patient with a hematocrit of 35 to 40. In general, 34 to 53 ml of blood will be required. The lower the hematocrit, the smaller the amount of blood required. The table includes tubes and volumes for biochemistry assays done at the Cental Biochemistry Laboratory. The volume indicated does not allow for rechecking any tests. Send all available serum from the number of tubes drawn.

Draw extra serum for duplicate quality control samples for the CBL and for the local laboratory 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" table of the manual (page 1.3.80-a) 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.

3.4.2 Labeling

All mailing tubes should be labeled with the patient's ID number, name code, date, and visit number. Urine aliquots must also have the total volume on the label. In addition, the GFR sample tubes are identified with specific sample designation labels (e.g., U1 for first timed urine sample). These labels will be provided by the Central GFR Lab.

Labeling All Specimens

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

3.4.3 Instructions for Drawing Blood for Biochemistry Samples

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 three minutes. Blood is drawn using the Vacutainer system following the instructions supplied with the system. A 1-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 completed filled, an extra tube of blood should be drawn and combined specimens sent. All Biochemistry samples and amino acid samples must be drawn prior to 125I-Sodium Iothalamate injection.

3.4.4 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 four-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 or 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 mls. 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.

3.4.4.1 Instructing the Patient on Collecting their 24 hour Urine

1. Review with the patient the 24 hour urine collection instructions on page 1.2.55 of the manual.
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.
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.

3.4.4.2 Acceptance of 24-Hour Urines

1. The 24 hour urine checklist (Appendix XVIII) 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 following criteria must be met:

   1. 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.
   2. Starting and ending date and time must be confirmed. The urine collection time must be within 23.5 and 24.5 hours.
   3. The patient should have emptied their bladder at the "start time" and discarded this sample.
   4. Every urine sample during the 24 hour time period must have been saved.
   5. The patient should have emptied their bladder at the "ending time" and saved this urine.
   6. The patient should not have had a short term illness during the collection.
   7. The patient should not have been menstruating during the collection.

8. The patient should have drank the usual amount of fluids and ate the usual amount of food during the collection period.
9. The jug should not have been frozen or overheated. Refrigeration during the collection is recommended.

3. Once the checklist is completed, it should be given to the dietitian. If the dietitian discovers any information which may indicate that the urine was improperly collected, they should write this information on the checklist and return it to the technician. 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.

3.4.5 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 which 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 on Form 17.
   DO NOT SUBTRACT THE AMOUNT OF THE ACID. The lab needs to know whether 250 ml or 500 ml were used. Enter amount of preservative on Form 17.
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.

3.4.6 Serum-for Biochemical analysis or for GFR samples.

Note: Remember that GFR Samples are radioactive and use equipment designed for RADIOACTIVITY.

1. Allow the blood to clot at room temperature for at least 30 minutes or up to 2 hours.
2. Spin down in a benchtop centrifuge.
3. Pour or pipet the serum into mailing tubes.
   a. GFR serum samples are sent in individual 5 ml red top mailing tubes.
   b. Serum for any combination of biochemistry tests is sent in a single 15 ml red top mailing tube. Refer to "Sample Requirements" table in Appendix VII for clarification of amount needed at each visit.
4. Close lids tightly.

3.4.7 Drawing Blood Samples and Processing Plasma for Amino Acid Analysis

The overall scheme for sample preparation is shown in Figure 1. Note, label all tubes required to process a sample with patient name, number, etc. before beginning!

1. Blood samples (minimum of 5 ml) are drawn from subjects under appropriate conditions. Blood samples should be obtained after an overnight fast and before infusion of $I^{125}$-label used for GFR studies. Please note that fasting includes abstaining from ingestion of the ketoacid preparation. Blood samples must be drawn into heparinized tubes (to prevent clotting), the stopper firmly replaced and the tube inverted 4 or 5 times (to dissolve the heparin). If a heparin lock is used to draw the blood sample, remember to draw sufficient blood through the "lock" to remove the heparinized saline in the lock (and discard this blood—saline mixture) prior to drawing the blood sample to be prepared for amino acid analysis.
2. The whole blood is prepared immediately, (or if this is impossible it is held on ice for no longer than 15 minutes before

centrifugation) by centrifuging at 2000 x G in a clinical centrifuge for 5 minutes to separate the erythrocytes (bottom layer) from plasma (upper layer). The plasma layer is carefully withdrawn and transferred to a labelled polystyrene tube using a Pasteur pipette (or other suitable pipette), taking special care to exclude the buffy coat layer (interface layer).
If clotting is a problem in a particular laboratory and/or a particular patient, the plasma layer may be removed to another heparinized tube rather than to the polystyrene tube provided. If this is done, make sure that the plasma is swirled gently to dissolve the heparin prior to withdrawing the two 1.00 ml samples from the pooled plasms for transfer to the tubes containing internal standards.

3. Using the Eppendorf or similar pipetting device, add exactly 1.00 ml of plasma to each of two labeled test tubes containing internal standards (obtained from the CAAL). One tube contains the internal standards with sodium dodecylsulfate (labeled SDS I.S.), the other tube contains only the internal standards (labeled I.S.). The tubes are capped, gently and repeatedly inverted for about 30 seconds, and then allowed to stand at room temperature for about 5 minutes.
4. The remaining plasma (extra plasma) is transferred to a purple bullet tube (supplied by the CAAL), labeled and frozen at -70 degrees C until shipped to the CAAL.
5. Assemble and label two Centricon-10 Microconcentrator devices (provided by the CAAL). Transfer the contents of each of the two tubes containing plasma and internal standards (with and without sodium dodecylsulfate) to separate labeled Centricon-10 Microconcentrator devices (provided by the CAAL).
The transfer of the contents of each of the two tubes containing plasma and internal standards to the ultrafiltration assemblies can be carried out using Pasteur pipettes; this transfer does not need to be quantitative.
6. Both microconcentrator assemblies are then centrifuged at room temperature in a fixed angle rotor at 3000-5000 x G until between

300 to 400 micro L of protein-free ultrafiltrate are obtained (about 30 min).

7. The top portion of each microconcentrator assembly is discarded. The filtrates obtained from the ultrafiltration of the plasma sample with and without sodium dodecylsulfate are transferred from the filtrate cups to the labeled, small, wide-mouthed, conical, plastic test tubes (supplied by the CAAL) and tightly capped.
8. The capped, labeled tubes are firmly tied together with a rubber band and then attached to the corresponding labeled purple bullet tube containing the extra plasma and stored at -70 degrees C until the 3 samples are sent as one unit on dry ice by Airborne Express to the CAAL at the following address: MDRD Central Amino Acid Laboratory, c/o Dr. L. D. Stegink, Department of Pediatrics, S-385 Hospital School, The University of Iowa, Iowa City, IA 52242. Be sure to include a completed MDRD Form 19 for each sample in the shipping container.

3.4.8 Whole Blood (EDTA)-for hemoglobin A1c analysis

1. Obtain 2 ml of EDTA blood. A 2 ml pediatric EDTA tube works especially well.
2. MIX WELL by inverting the tube 4-5 times immediately before pouring off.
3. POUR the blood directly from EDTA vacutainer tubes (purple top) into 7 ml green top mailing tubes. (A 2 ml tube may be placed directly inside the green mailing tube.)
4. CLOSE the tube tightly.

3.4.9 Sample Storage

The GFR samples may be batched and sent once a week. If you want your GFR results in a more timely fashion, the samples can be sent more often. All CBL specimens (serum, whole blood and 24 hour urine aliquots), should be mailed the same day, if possible. The only exception to this is 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, with the exception of tubes for amino acids, should be refrigerated until they are packed with freezer packs in the styrofoam mailers.

Prepared plasma samples for amino acid analysis are to be stored at -70 degrees C at each Clinical Center until shipped on dry ice by Airborne Express to the Central Amino Acid Laboratory once a month or when the Clinical Center has accumulated 25 samples, which ever comes first. The cost of the dry ice needed for shipping samples from the Clinical Centers to the Central Amino Acid Laboratory must be paid by each individual Clinical Center.

3.4.10 Sample Mailing Instruction

Make sure to send all GFR samples and accompanying forms to the Central GFR Lab, and all CBL samples and accompanying forms to the CBL Lab. Place all mailing tubes into zip lock bags as follows: (1) all GFR samples (i.e., all radioactive samples plus backgrounds) will be placed in a ziplock bag and shipped separately from any biochemistry samples, and (2) all biochemistry samples will be sealed in a separate zip lock bag. Place two paper towels in these bags to absorb any leakage that might occur. Attach a piece of yellow/magenta tape with the message 'radioactive' on the outside of the bag containing the GFR samples. 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 (e.g., Form #16, and/or Form #17) 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 central lab GFR samples should be sent by a next-day mail service to the GFR Central Laboratory address. All CBL samples should be sent by a next day mail service to the CBL address.
Amino acid samples should be mailed to the CAAL by Airborne Express using the mailing boxes and preaddressed Airborne Express airbills supplied to the Clinical Centers by the CAAL. Prepared samples are to be shipped on dry ice (preferably 10 pounds, which will fill the box, but no less than 5 pounds) to the CAAL once a month or when the Clinical Center has accumulated 25 samples, which ever comes first. The cost of the dry ice needed for shipping samples must be paid by each individual center. Do not send samples on a Friday or the day before a holiday. Remember to enclose the CAAL's copy of Form 19.

# Chapter 3, Part 4
# Quality Control

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## 4.1 Overview of Laboratory Quality Control

### Clinical Center Laboratories

4.1.1 Clinical Center laboratories will be monitored in the following way

1. CAP survey results will be provided on a yearly basis to the Principal Investigator of the CBL for review.
2. The following tests will be assessed for quality control.

Serum:

| | |
|---|---|
| Magnesium | (mg/dl) |
| Calcium | (mg/dl) |
| Urea Nitrogen | (mg/dl) |
| Creatinine | (mg/dl) |

4.1.2 The Central Biochemistry Laboratory will be monitored in two ways.

1. External Quality Control. Split patient samples (one labelled with the patient ID and one labelled with the QC ID) from the Clinical Centers will be used to monitor reproducibility.
2. External Quality Control (CAP Samples). These QC Samples (with known ranges for each constituent) will be used to monitor accuracy. Further details follow.
3. The following tests will be quality controlled:

Serum:

Albumin, Transferrin*, Phosphorus, Urea Nitrogen, Creatinine, LDH, SGOT, Bilirubin, Uric Acid

Lipid Profile:

T. Cholesterol, Triglyceride, HDL-Cholesterol

Urine:

Protein, Phosphorus, Urea Nitrogen, Creatinine, Sodium, Potassium

Whole Blood:

Hemoglobin A1c*

* CAP Samples are not available for transferrin or Hemoglobin $A1_c$.

Procedures for External Quality Control of the Central Biochemistry Laboratory are in Section 4.4.

4.1.3 Central Amino Acid Laboratory

Internal quality control of the Central Amino Acid Laboratory is as follows: The between sample variability of the amino acid analyses, as a result of day-to-day changes in the ninhydrin, are assessed by the inclusion of a standard sample of deproteinized plasma with each batch of patient samples analyzed. This standard sample is an aliquot of a large volume of simulated plasma and stored at -70 degrees in small aliquots. Runs of samples in which the amino acid totals of this standard sample differ from the mean totals for this standard by more than +/-10% are reanalyzed until the standard sample values are within this range.

For external quality control, each Clinical Center Lab performs quality control of the Central Amino Acid Lab once annually. The Clinical Center lab technician draws additional blood from a patient, prepares it, splits it, and sends the two specimens to the Central Amino Acid Lab. One of the samples will be labelled with the QC ID and name code. The DCC will suggest which patient's samples should be used. Duplicate results are compared. This is further described in Section 4.5.

For sample preparation quality control at each Clinical Center:

1. It is critical that sample preparation at the Clinical Centers be accurate. To check this point, we will submit a simulated plasma sample of known composition and containing 10 $\mu$-moles/dL added alloisoleucine as internal standard to each Clinical Center both prior to the start of the study and every 6 months thereafter. The Clinical Centers will prepare the simulated plasma and return the prepared solutions to the Central Amino Acid Laboratory (see Figure 2). Systematic errors in sample preparation will be detected by calculation of S-aminoethylcysteine and $\alpha$-methyltryptophan concentration using alloisoleucine concentration as the internal standard.

2. After performing an amino acid analysis on the prepared simulated standard plasma samples from each Clinical Center, Dr. Stegink and Mr. Brummel will review the results and compare the amino acid concentrations observed to actual concentrations present in the standard.
3. Results of analyses in which amino acid concentrations are within acceptable limits will be transmitted to the DCC using Datalex form 36.
4. For results outside of the acceptable limits the following action will be taken:
   a. The Clinical Center will be informed that the results fell outside acceptable limits.
   b. The CAAL will mail out another quality control sample for preparation and return to the laboratory.
5. After the second sample has been prepared, returned to the CAAL and analyzed, the results will again be reviewed by Dr. Stegink and Mr. Brummel. If the problem has been corrected and the results now fall within acceptable limits, no further action is necessary. If the second set of results also fall outside of the acceptable limits the following actions will take place:
   a. The Clinical Center involved will be informed that the second set of values are outside of acceptable limits.
   b. Dr. Stegink will inform the Chairman of the Quality Control Committee in writing of the problem. The Chairman will review the problem with the Committee.
   c. The Quality Control Committee will work with the Clinical Center Principal Investigator to resolve the problem.

For <u>duplicate</u> samples from Clinical Centers:

1. Every 6 months, each Clinical Center will send one duplicate sample to the CAAL. The CAAL will analyze the samples and compare the results. This is explained in greater detail in Section 4.5.
2. The data will be entered on Datalex form 36 and transmitted to the DCC.
3. Review of results:
   a. The CAAL will prepare a report comparing the duplicate quality control results.
   b. The report will be sent to the Quality Control Committee.

c. Results which vary by an absolute difference of more than 10% CV will be reviewed by the principal investigator of the CAAL and repeat analyses will be carried out if sample is available.

4.1.4 Central GFR Laboratory

Internal Quality Control of the Central GFR Lab is accomplished as follows:

1. The pipettor used for GFR samples is routinely evaluated for volumetric accuracy and precision at six month intervals using the weighing of water on an electronic microbalance as a quality control technique.
2. The gamma counter is calibrated with 137Cesium standards to assure accurate peak locations and window settings.
3. The counter efficiency is monitored daily using 137 Cesium standards. Counter background activity is monitored on a daily basis as well.
4. The patient counts are bracketted by matched Glofil standards to eliminate instrumental malfunctions during sample counting as an error source.
5. A precision study is run weekly by randomly selecting a GFR study and rerunning it. External Quality control of the Central GFR Laboratory will be carried out as follows:

Whenever a clinical center sends GFR specimens to the Central GFR Laboratory, backup specimens should be saved. Every three months, each clinical center will perform the following procedure for one GFR:

After the GFR results come back from the Central Lab, the cinical center technician will prepare a patient's backup specimens for mailing, using that center's "quality control identification number and name code" on the tubes and on the Mailing form.

The Data Coordinating Center will suggest which patient's backup specimens should be used. (The MDRD technician should use the visit number from the

original GFR.) The Clinical Center technician will inform the Data Coordinating Center which patients' GFR the quality control GFR should match using the Central Lab QC ID Matching Form 22. Results from the first GFR and the second GFR will be compared for quality control.

4.2. **External Quality Control for Clinical Center Laboratories**

The CBL will co-ordinate and monitor the external QC program for the local laboratories.

4.2.1 Request of Results

The CBL will request a copy of each local laboratory's CAP survey result once each year for review by the Principal Investigator of the CBL.

4.2.2 Review of Results

The following tests will be reviewed:

1) Magnesium
2) Calcium
3) Urea Nitrogen
4) Creatinine

4.2.3 Out-of-Range Results

Any unacceptable results will be reported to the Quality Control Committee. Results of the next CAP survey will then be requested for review.

4.2.4 Continuing Problems

Any on-going problems with a particular constituent will be brought to the attention of the Quality Control Committee Chairman.

4.3 External QC for Central Biochemistry Laboratory by the Clinical Center Laboratories. The submission of duplicate specimens to the CBL will monitor on-going precision of the CBL. Results of comparisons will be reported by the Data Coordinating Center.

4.3.1 Timing and Patient Selection

1. Twice yearly, each clinical center will send one set of duplicate quality control samples to the CBL. The schedule will be sent to the centers from the DCC.
2. Duplicate QC samples should be drawn from patients who are having a Baseline 3 month visit, or an 8 or 16 month follow-up visit (or other multiple of 8) in order to monitor precision of all tests run at the CBL. If one of these visits is not due, a 4-month follow-up is the next best to use.

4.3.2 Amount Required

1. Draw two additional 10 ml SST vacutainer tubes, or other type tubes which have no anticoagulant in them. The minimum amount of serum needed is 7 ml.
2. Extra EDTA tubes for the hemoglobin A1c need not be drawn if the original tube is filled completely when drawing. Hemoglobin $A_{1C}$ analysis requires 1 ml of whole blood.

4.3.3 Sample Processing

1. Label one set of mailing tubes with the QC ID and name code. Label a duplicate set of tubes with the patient information. Split the urine sample by mixing the entire 24 hour collection well and aliquoting two tubes with approximately 25 mls in each. Split the serum sample by mixing well and pouring at least 7 ml into the "QC" tube, and at least 13 ml into the patient tube. Put tubes in a ziplock bag.
2. Split the Hemoglobin sample by mixing well and placing 2 ml whole blood into each of 2 green top mailing tubes (one with blank label for QC, and one with patient code name and number).
3. The CBL will receive these samples and handle them in the same manner as a patient sample. Form 32 and 33 are completed as for a regular patient.

4.4 External QC for the Central Amino Acid Lab (CAAL)

4.4.1 Timing

Twice a year, the local on site Clinical Center Laboratory

technicians will draw an additional blood sample from a patient, prepare it, label each sample, and send both specimens to the Central Amino Acid Laboratory for analysis. Duplicate results will be compared.

4.4.2 Sample Processing

The overall scheme for sample preparation is shown in Figure 3. Note, label all tubes required to process a sample with patient name, number, etc., before beginning.

1. Call the DCC to obtain the QC ID code name and number for the patient selected to serve as the quality control sample.
2. A heparinized blood sample (minimum of 10 ml) is drawn from the designated subject under appropriate conditions. Blood samples should be obtained after an overnight fast and before infusion of $I^{125}$-label used for GFR studies. Please note that fasting includes abstaining from ingestion of the ketoacid preparation.

   The blood sample is drawn into a heparinized tube (green top), the stopper firmly replaced and the tube inverted 4 or 5 times (to dissolve the heparin). If a heparin lock is used to draw the blood sample, remember to draw sufficient blood through the "lock" to remove the heparinized saline in the lock prior to drawing the blood sample to be prepared for amino acid analysis.

   The whole blood is prepared immediately, (or if this is impossible it may be held on ice for no longer than 15 minutes before centrifugation) by centrifuging at 2000 x G in a clinical centrifuge for 5 minutes to separate the erythrocytes (bottom layer) from plasma (upper layer). The plasma layer is carefully withdrawn and transferred to a labelled polystyrene tube using a Pasteur pipette (or other suitable pipette), taking special care to exclude the buffy coat layer (interface layer).

   If clotting is a problem in a particular laboratory and/or a particular patient, the plasma layer may be removed to another heparinized tube rather than to the polystyrene tube provided.

If this is done, make sure that the plasma is swirled gently to dissolve the heparin prior to withdrawing samples from the pooled plasma for transfer to the tubes containing internal standards.

3. Using the Eppendorf or similar pipetting device, add exactly 1.00 ml of plasma to each of four labeled tests tubes containing internal standards (obtained from the CAAL). Two of the tubes used should contain the internal standards with sodium dodecylsulfate (labeled I.S.). The other two tubes used should contain the internal standards without sodium dodecylsulfate (labeled I.S.). The tubes are capped, gently and repeatedly inverted for about 30 seconds (or till the SDS is completely dissolved), and then allowed to stand at room temperature for about 5 minutes.
4. The remaining plasma (extra plasma) is divided into two equal parts and the two halves are placed in individual, appropriately-labeled, purple "Bullet Tubes" (labeled either with the regular patient number or with the quality control number assigned by the DCC). The two tubes are stored frozen at -70 degrees C until shipped to the CAAL attached to the corresponding prepared sample tubes.
5. Assemble and label four Centricon-10 Microconcentrator devices. Transfer the contents of each of the four tubes containing plasma and internal standards (two with and two without sodium dodecylsulfate) to separate labeled Centricon-10 Microconcentrator devices (provided by the CAAL).
6. All four microconcentrator assemblies are then centrifuged at room temperature in a fixed angle rotor at 3000-5000 x G until between 300 to 400 UL of protein-free ultrafiltrate are obtained (about 30 min).
7. The top portion of each microconcentrator assembly is discarded. The filtrates obtained from the ultrafiltration of the plasma samples with and without sodium dodecylsulfate are transferred from the filtrate cups to the labeled, small, wide-mouthed, conical, plastic test tubes (supplied by the

CAAL) and tightly capped. Note, that as an alternative the filtrate cups themselves may be capped, labeled and sent to the CAAL, eliminating the need to transfer the sample to the small, wide-mouthed, conical test tubes.

8. Each of the two sets of capped, labeled tubes (2 tubes/set; SDS) is firmly tied together with a rubber band and then attached to the corresponding labeled test tubes containing the extra plasma and stored at -70 degrees C until the two sets of 3 tubes each are sent on dry ice to the CAAL. Alternatively, the sample sets can be kept together in small plastic zip-lock bags, eliminating the need for rubber bands. Prepared external control samples are to be shipped on dry ice by Airborne Express to the Central Amino Acid Laboratory as soon as possible.

   Be sure to include a completed MDRD Form 19 for each sample in the shipping container (both the original sample and the duplicate).

   Be sure not to ship samples when the next day is a weekend or a holiday.

4.4.3 Results

Results of the comparisons will be reported by the Data Coordinating Center.

2. Split the Hemoglobin sample by mixing well and placing 2 ml whole blood into each of 2 green top mailing tubes (one with blank label for QC, and one with patient code name and number).
3. The CBL will receive these samples and handle them in the same manner as a patient sample. Form 32 and 33 are completed as for a regular patient.

## 4.5 External QC for the Central Amino Acid Lab (CAAL)

### 4.5.1 Timing

Twice a year, the local on site Clinical Center Laboratory technicians will draw an additional blood sample from a patient, prepare it, label each sample, and send both specimens to the Central Amino Acid Laboratory for analysis. Duplicate results will be compared.

### 4.5.2 Sample Processing

The overall scheme for sample preparation is shown in Figure 3. Note, label all tubes required to process a sample with patient name, number, etc., before beginning.

1. Call the DCC to obtain the QC ID code name and number for the patient selected to serve as the quality control sample.
2. A heparinized blood sample (minimum of 10 ml) is drawn from the designated subject under appropriate conditions. Blood samples should be obtained after an overnight fast and before infusion of $I^{125}$-label used for GFR studies. Please note that fasting includes abstaining from ingestion of the ketoacid preparation.

   The blood sample is drawn into a heparinized tube (green top), the stopper firmly replaced and the tube inverted 4 or 5 times (to dissolve the heparin). If a heparin lock is used to draw the blood sample, remember to draw sufficient blood through the "lock" to remove the heparinized saline in the lock prior to drawing the blood sample to be prepared for amino acid analysis.

The whole blood is prepared immediately, (or if this is impossible it may be held on ice for no longer than 15 minutes before centrifugation) by centrifuging at 2000 x G in a clinical centrifuge for 5 minutes to separate the erythrocytes (bottom layer) from plasma (upper layer). The plasma layer is carefully withdrawn and transferred to a labelled polystyrene tube using a Pasteur pipette (or other suitable pipette), taking special care to exclude the buffy coat layer (interface layer).

If clotting is a problem in a particular laboratory and/or a particular patient, the plasma layer may be removed to another heparinized tube rather than to the polystyrene tube provided. If this is done, make sure that the plasma is swirled gently to dissolve the heparin prior to withdrawing samples from the pooled plasma for transfer to the tubes containing internal standards.

3. Using the Eppendorf or similar pipetting device, add exactly 1.00 ml of plasma to each of four labeled tests tubes containing internal standards (obtained from the CAAL). Two of the tubes used should contain the internal standards with sodium dodecylsulfate (labeled I.S.). The other two tubes used should contain the internal standards without sodium dodecylsulfate (labeled I.S.). The tubes are capped, gently and repeatedly inverted for about 30 seconds (or till the SDS is completely dissolved), and then allowed to stand at room temperature for about 5 minutes.
4. The remaining plasma (extra plasma) is divided into two equal parts and the two halves are placed in individual, appropriately-labeled, purple "Bullet Tubes" (labeled either with the regular patient number or with the quality control number assigned by the DCC). The two tubes are stored frozen at -70 degrees C until shipped to the CAAL attached to the corresponding prepared sample tubes.
5. Assemble and label four Centricon-10 Microconcentrator devices. Transfer the contents of each of the four tubes containing plasma and internal standards (two with and two

without sodium dodecylsulfate) to separate labeled Centricon-10 Microconcentrator devices (provided by the CAAL).

6. All four microconcentrator assemblies are then centrifuged at room temperature in a fixed angle rotor at 3000-5000 x G until between 300 to 400 UL of protein-free ultrafiltrate are obtained (about 30 min).

7. The top portion of each microconcentrator assembly is discarded. The filtrates obtained from the ultrafiltration of the plasma samples with and without sodium dodecylsulfate are transferred from the filtrate cups to the labeled, small, wide-mouthed, conical, plastic test tubes (supplied by the CAAL) and tightly capped. Note, that as an alternative the filtrate cups themselves may be capped, labeled and sent to the CAAL, eliminating the need to transfer the sample to the small, wide-mouthed, conical test tubes.

8. Each of the two sets of capped, labeled tubes (2 tubes/set); SDS) is firmly tied together with a rubber band and then attached to the corresponding labeled test tubes containing the extra plasma and stored at -70 degrees C until the two sets of 3 tubes each are sent on dry ice to the CAAL. Alternatively, the sample sets can be kept together in small plastic zip-lock bags, eliminating the need for rubber bands. Prepared external control samples are to be shipped on dry ice by Airborne Express to the Central Amino Acid Laboratory as soon as possible.

   Be sure to include a completed MDRD Form 19 for each sample in the shipping container (both the original sample and the duplicate).

   Be sure not to ship samples when the next day is a weekend or a holiday.

4.5.3 Results

Results of the comparisons will be reported by the Data Coordinating Center.

# Chapter 3, Part 5
# Blood Pressure Measurement, Training and Quality Control

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## 5. Introduction

### 5.1 Overview

In the MDRD Study, sitting blood pressure is measured monthly in a resting state, using three measurements with a random-zero sphygmomanometer. The random-zero device has an important advantage over the conventional fixed zero manometer: it prevents the MDRD blood pressure observer from knowing the actual blood pressure value and therefore removes judgments about blood pressure levels for readings close to critical values such as a diastolic of 90 mm Hg.

Correct measurement of blood pressure is of the utmost importance to the success of this study because blood pressure measurement is one of the two primary interventions of the study. It is essential that the procedures described below for measuring blood pressure be followed exactly. Precision is essential for valid comparisons of blood pressures between treatment groups and in individuals across time.

### 5.2 Background

Experience in providing training support for blood pressure observers, to help them achieve and maintain a high standard of measurement performance, has been a potentially valuable by-product of the nation-wide Hypertension Detection and Follow-up Program (HDFP)[1] and the Systolic Hypertension in the Elderly Program (SHEP)[2]. This training activity was necessarily an integral aspect of HDFP and SHEP. However, the measurement problems addressed in the Programs were not unique, and the solutions formulated by the investigators of these trials may therefore be helpful to others who encounter some of the same problems in planning detection and/or follow-up procedures for high blood pressure.

In this spirit, we present to the MDRD investigators the essential components of the HDFP/SHEP training and certification programs for blood pressure observers. These components include the step-by-step

procedures for use of the random-zero sphygmomanometer; brief lecture/slide presentations for initial orientation of trainees; and the training/certification procedure developed for the HDFP, adapted for the SHEP, and now adapted for use in the MDRD Study including a videotape test for the quantitative assessment of individual observers' measurement performance. The actual scoring for this videotape test and its procedures will be conducted by the Data Coordinating Center.

It should be noted that other reference materials are available which blood pressure observers would be well advised to consult. Foremost is the American Heart Association booklet, "Recommendations for Human Blood Pressure Determination by Sphygmomanometers" (the latest edition is by Frolich, et al 1987)[3] which has long been regarded as a standard reference on the subject. In addition, a 1978 publication by Prineas (Blood Pressure Sounds: Their Measurement and Meaning - A Training Manual)[4] provides a comprehensive discussion of the problems in blood pressure measurement as well as an extensive bibliography.

## 5.3 Training and Certification

High quality blood pressure readings are fundamental to any sound program measuring and controlling blood pressure levels.[6-8] Yet many factors, including influences of the subject, the observer, the equipment, and the circumstances of measurement, work against the attainment of this basic objective. Thus, good results cannot be taken for granted and special attention must be focused on blood pressure measurement procedures.

Before the actual initiation of standardized measurements, a program of training and certification must be provided so that all staff responsible for recording blood pressure readings will be certified as having met a stipulated level of performance. Recertification will be required at annual intervals for the duration of an observer's service in MDRD.

The MDRD certification process includes training and the successful completion of:

* a written test
* a live evaluation
* a videotape test.

The training strategy adopted by MDRD is a two-stage program. Before the program begins, each Clinical Center will identify one specific Training Supervisor for that clinic. These Training Supervisors from each Clinical Center (and other blood pressure observers, if the Centers desire) will meet centrally in October 1989 for the first stage of training. The full training program will be presented at this time. The supervisors and observers who pass the program will be certified as Blood Pressure Observers. The Supervisors can, in turn, train additional observers in the Clinical Centers. This is the second stage of training. To this end, each Center will be provided with the full set of training materials needed to reproduce the same program for their field and clinic staff. In this second stage, the Data Coordinating Center will receive documentation of each observer's training performance from the Training Supervisors in the Clinical Centers (including the successful completion of the written test and the live evaluation). However, scoring of the video test will be done by the Data Coordinating Center, WHICH IS RESPONSIBLE FOR IDENTIFYING WHO IS A CERTIFIED MDRD BLOOD PRESSURE OBSERVER. Results of the certification tests will be telephoned (and subsequently mailed) to a Clinical Center within three (3) working days of receipt of the test data from that clinic. Through this scheme, training will be the responsibility of both the Clinical Centers and the Data Coordinating Center. The Data Coordinating Center will, in addition, remain responsible for overall monitoring and quality control (as will be described in Section 5.7).

### 5.4.1 Overview

In this chapter, the step-by-step procedures for blood pressure measurement in MDRD are presented. It should be emphasized that the steps outlined here can satisfactorily be followed for the vast majority of adult subjects participating in ambulatory screening and follow-up. Exceptional situations do arise, with sometimes serious obstacles to successful blood pressure measurement. The training program for a particular setting must include guidelines for handling such exceptions. Only a few will be noted. It will be the responsibility of the Training Supervisors in the Clinical Centers and in the Data Coordinating Center to encourage observers to note exceptional circumstances and to seek consultation with the Blood Pressure Expert at the DCC when they arise so that participants will be appropriately evaluated.

Standardized Hawksley random-zero (R-Z) sphygmomanometers are used for blood pressure measurement for all MDRD clinic visits and for the determination of peak inflation levels. This manual will concentrate on this device. Because the conventional Baum sphygmomanometer may be used in screening, this device will also be described to a lesser extent, but the procedures are identical (except, of course, for resetting and recording the random-zero level).

It should be noted also that the procedures listed here are illustrated in the third lecture/slide presentation, "Procedures in Blood Pressure Recording" (Section 5.5.7).

### 5.4.2 Preparation for Blood Pressure Measurement

Some of the many extraneous factors influencing blood pressure are controlled by standardizing the measurement technique and the environment in which the measurement is made. Uncontrolled factors (time of day, identity of the observer) are recorded, so that they can be taken into account during analysis.

MDRD patients must abstain from caffeine and smoking at least one half hour prior to and until completion of the blood pressure measurement. Current drug intake, including medications affecting blood pressure and non-prescription drugs, is recorded on the day of the examination.

Try to keep the blood pressure measurement as pleasant as possible. Patients should be given full explanation and instructions about the preparation for the blood pressure examination and an opportunity for brief questions. The setting in which blood pressure measurements are made will be standardized, and should take place in a separate, quiet room where no other activity is taking place, and where temperature fluctuations are minimal. Scheduling procedures should try to establish consistent appointment times to minimize as much as possible the impact of daily blood pressure variation. Equipment (including study forms, sphygmomanometer, etc.) should be checked and waiting for the participant.

Allow five minutes of rest in this quiet room after arm measurement and calculation of corrected peak inflation level but before taking the blood pressure. Explain to the participant that the five minute rest period will provide for more valid blood pressure measurements. Try to keep conversations with the participant to a minimum at this time. The observer should either leave the room or sit quietly with the participant. The participant should be relaxed, seated with legs uncrossed and feet comfortably flat on the floor, not dangling.

### 5.4.3 Measurement Procedures

The sitting arm blood pressure is measured three times at each clinic visit. It takes approximately 10 to 15 minutes to make three blood pressure measurements including the initial five minute rest. The blood pressure measurements should be done early in the clinic visit but blood pressure measurements are not to be done while a GFR or any other procedure is being performed.

Blood pressure equipment should be checked prior to seeing the patient. Once a participant is given instructions and explanations

blood pressure measurement begins. The following steps must be followed precisely. The procedure is described here employing the MDRD Blood Pressure Form 46.

### 5.4.4 Stethoscope

A standard Littman stethoscope (or other comparable stethoscope) with a bell is used. Korotkoff sounds are best heard with the bell because of their low pitch. Stethoscope tubing should be about 10 to 12 inches from the bell piece to "Y" branching. This length provides optimal acoustical properties and allows the observer to read the sphygmomanometer at eye level and in a comfortable position. Earpieces should fit comfortably and snugly in the ears. Four points should be observed in using the stethoscope.

1. The ear pieces should be directed forwards into the external ear canal.
2. The ear pieces should be tight enough to exclude outside sound but not so tight that they cause discomfort.
3. The valve between the bell and the diaphragm should be turned in the direction of the bell.
4. The bell of the stethoscope should be placed lightly on the skin overlying the brachial artery - immediately below, but not touching, the cuff. The brachial artery is usually found above the crease of the arm, slightly towards the body. Light pressure accentuates low-pitched sound and avoids compression murmurs. Pressing too heavily with the stethoscope over the brachial artery causes turbulent flow in the artery and a murmur can be heard which may prolong the apparent duration of fourth-phase Korotkoff sounds.

### 5.4.5 Arm Measurement and Cuff Sizes

The proper cuff size must be used to avoid under- or over-estimating the correct blood pressure. To determine the proper cuff size, the observer must measure the arm circumference at the midpoint of the arm. This measurement is taken on the right arm that has been bared from the shoulder. With the participant standing, holding the forearm horizontal, the arm length is measured from the acromion (or boney

extremity of the shoulder girdle) to the olecranon (or tip of the elbow) with a metric tape. The midpoint is marked on the dorsal surface. The participant should then relax the arm along the side of the body. The arm circumference is measured by drawing the tape snugly around the arm at the level of the midpoint marking. Care must be taken to keep the tape horizontal. Also, the tape should not indent the skin. The chart of arm circumference measurements and corresponding cuff sizes (shown below) is consulted, and the indicated cuff size is checked on the study form and used. Do not use the cuff itself as a measurement device because the ranges marked on the cuff may not correspond with the table. A copy of this chart should be attached to the sphygmomanometer for easy reference. This chart should be consulted for each arm measurement. The markings found on most blood pressure cuffs should not be used for reference because they may be incorrect.

Determination of Cuff Size Based on Arm Circumference

| Arm Circumference | Cuff Size (cm) |
|---|---|
| < 24 cm | Child, Pediatric, Small Adult |
| 24 to 32 cm | Adult, Regular |
| 33 to 41 cm | Large |
| > 41 cm | Thigh, Extra Large |

---

### 5.4.6 Application of the Blood Pressure Cuff

Next, the appropriate cuff (as determined in the arm measurement procedure) is placed around the upper right arm so that the midpoint of the length of the bladder lies over the brachial artery and the mid-height of the cuff is at heart level. The lower edge of the cuff, with its tubing connections, should be placed about 1 inch above the natural crease across the inner aspect of the elbow. The cuff is wrapped snugly about the arm, with the palm of the participant's hand turned upward. The wrapped cuff should be secured firmly by applying pressure to the locking fabric fastener over the area where it is applied to the cuff.

### 5.4.7 Determining the Peak Inflation Level

For each participant it is necessary to determine the pressure level to which the cuff is to be inflated for accurate measurement of the systolic pressure. This is because the pressure at the start of the reading should always exceed the systolic pressure, otherwise the first of the Korotkoff sounds will be missed (see Lecture #1). This starting pressure is called the Peak Inflation Pressure and is determined as follows. First, the cuff tubing should be attached to the random-zero sphygmomanometer. With the control valve in the OPEN position, the cuff should be inflated while the radial pulse is palpated and the mercury column watched closely. When sufficient pressure has been applied, the pulse will no longer be felt. The cuff should continue to be inflated until it reaches a level of 200 mm Hg. After 5 seconds (a slow count to 5) at 200 mm Hg, the control valve is turned to the CLOSE position. Feel for the pulse. If the pulse is still felt, the cuff pressure should be increased until the pulse disappears. Either the first or the second of these procedures will identify the Observed Pulse Obliteration Pressure. When this has been detected, the cuff is quickly and completely deflated. The Observed Pulse Obliteration Pressure and the zero-value are recorded in the spaces provided on the study form and the Corrected Pulse Obliteration Pressure is calculated. To the corrected value, add the R-Z maximum zero number for this device (found next to the mercury column) plus 20 mm Hg. This summed value is the Peak Inflation Level. The cuff is to be inflated to this level or to 200 mm Hg, whichever is greater, for all readings at this examination.

NOTE: All readings on the sphygmomanometer are made to the nearest even digit. Any reading that appears to fall exactly between markings on the column should be read to the next marking immediately above, i.e., 2, 4, 6, 8, or 0. All readings are to be made at the top of the meniscus, or rounded surface of the mercury column. Be careful: when the pressure is released quickly from a high level, a vacuum is formed

above the mercury and the meniscus is distorted. A few moments should be allowed for it to reappear before the manometer is read.

### 5.4.8 Pulse Measurement

Part of the blood pressure measurement procedure is the measurement of the pulse, as observed by palpation of the radial artery at the wrist. For simplicity, the right arm is to be used consistently for measurement of both pulse and blood pressure. This measurement serves two purposes: (1) to document the resting heart rate at the time of examination, and (2) to permit detection of gross irregularities of heart rhythm which may affect the interpretation of the blood pressure readings.

A good stop watch should be used for pulse measurements. The Cronus Stop Watch, Model 3-S, is an interval timer and is a preferred timing device. Of the various options, it seems to be the simplest and easiest to read and is generally available at a local sporting goods store. The address of the manufacturer is:

Cronus Precision Products, Inc.
2895 Northwestern Parkway
Santa Clara, California 95051

The measurement of pulse is performed only after the participant has been seated quietly, with feet flat on the floor, in an erect but comfortable posture, for at least five minutes. The patient should refrain from caffeine and smoking at least one half hour prior to and until completion of blood pressure measurement. The elbow and forearm should rest comfortably on the table. With the palm of the hand turned upward, the radial pulse is palpated and counted for 30 seconds exactly. The number of beats in 30 seconds is recorded, multiplied by 2, and the product recorded as the heart rate. Any marked irregularity observed during this period should be called to the attention of the Principal Investigator and the Blood Pressure Training Supervisor.

### 5.4.9 Blood Pressure Readings

Next, the observer should proceed to carry out the first blood pressure reading. Detailed instructions are given below for measuring

blood pressure with both a conventional and a random-zero sphygmomanometer.

### 5.4.10 The Random-Zero Mercury Sphygmomanometer

The design and operation of a conventional sphygmomanometer are based upon the combined principles of compression of the brachial artery under an elastic inflatable cuff, direct auscultation of the Korotkoff sounds through a standard stethoscope, and direct registration of pressure levels by a mercury manometer. The observer inflates the cuff, listens for the first- (systolic) and fifth- (diastolic) phase Korotkoff sounds, reads the mercury level in the column, deflates the cuff, and records the readings.

The Hawksley random-zero sphygmomanometer is also a mercury sphygmomanometer, with the same basic principles of operation. The essential distinction is a mechanism designed to produce a variable level of mercury in the manometer column when the actual pressure in the cuff is zero. This is accomplished through an adjustable-volume chamber which is interconnected with the mercury reservoir at one end and the manometer column at the other end. The adjustment is made by the observer by spinning an external wheel which contacts and rotates an internal, bevelled cam. The position where the cam comes to rest after spinning determines where the bevelled edge will meet the sliding wall of the mercury chamber. When air pressure is applied through the cuff, the wall is displaced until it rests against the cam and only the mercury remaining after filling this new volume of the chamber is displaced into the manometer column. A valve controlled by the observer locks the chamber system after the maximum inflation pressure desired has been applied so that at the end of the reading, and only at the end, the mercury comes to rest at its "randomly" determined zero-pressure level. When this value is subtracted from the recorded readings, the corrected readings give the corresponding actual pressure levels. Thus, by the addition of this mechanism for varying the "zero" level of mercury to the conventional device, the

random-zero device obscures the true levels of pressure observed until after the uncorrected blood pressure is recorded and the "zero" level is read and subtracted. In this way, some of the recognized difficulties in observer performance are substantially reduced, primarily interference by observer bias when readings fall near critical levels of blood pressure.

### 5.4.11 Measuring Blood Pressure with a Random-Zero Device

The steps for readings with the random-zero device are described below.

(1) Wait at least 30 seconds after complete deflation of the cuff following any preceding inflation.

(2) Connect the cuff to the random-zero device.

(3) Ensure that the mercury reservoir lever is in the operating position by turning the control valve on the face of the device to the right to the position marked OPEN.

(4) Using downstrokes only, gently turn the wheel at the right side of the device by stroking it a few times with the extended fingertips of the right hand. (Do not try to spin it wildly!)

(5) Place the ear pieces of the stethoscope into the ears, with the tips turned forward.

(6) Apply the bell of the stethoscope over the brachial artery, just below but not touching the cuff or tubing. The brachial artery is usually found at the crease of the arm, slightly toward the body.

(7) Using the previously determined peak inflation level, inflate to this level or to 200 mm Hg, whichever is greater. The eyes of the observer should be level with the mid-range of the manometer scale and focused at the level to which the pressure will be raised.

(8) By closing the thumb valve, hold the pressure at this level for 5 seconds (count to 5 slowly), and then turn the control valve to the left, to the position marked CLOSE.

(9) By opening the thumb valve slightly and maintaining a constant rate of deflation at approximately 2 mm per second, allow the cuff to deflate, listening throughout the entire range of deflation, from the maximum pressure past the systolic reading (the pressure where the first regular sound is heard), until 10 mm Hg below the level of the diastolic reading (that is, 10 mm Hg below the level where the last regular sound is heard).

(10) Open the thumb valve fully and disconnect the tubing from the cuff allowing the mercury to fall to its "zero" level for this reading. Remove the stethoscope earpieces from the ears.

(11) Record the uncorrected systolic and diastolic readings.

(12) Read the "zero" level for this reading and record it in the two spaces provided on the study form, beneath the uncorrected systolic and diastolic readings.

(13) By subtraction, calculate and record the actual systolic and diastolic readings in the spaces provided. These are the First Random-Zero Blood Pressure Values.

(14) Repeat steps 3 through 13 two more times, waiting at least 30 seconds after complete deflation of the cuff following any preceding inflation. These are the Second and Third Random-Zero Blood Pressure Values.

### 5.4.12 Criteria for Systolic and Diastolic Blood Pressure

To correctly identify the 1st-phase (systolic) and 5th-phase (diastolic) Korotkoff values, the observer must listen carefully via the stethoscope while reading and interpreting the mercury column. The systolic value can be identified as the pressure level where the first of 2 or more sounds are heard in appropriate rhythm. The diastolic value can be identified as the pressure level where the last of these rhythmic sounds is heard (that is the last audible rythmic sound not the first point where sound is not heard). The mercury should be made to drop at 2 mm Hg per second, from the maximum pressure until 10 mm Hg below that of the last regular sound heard. The control of the deflation rate is essential for accurate readings and depends on handling of the bulb and its control valve.

PLEASE NOTE: A single sound heard in isolation (i.e., not in rhythmic sequence) before the first of the rhythmic sounds (systolic) or following the last of the rhythmic sounds (diastolic) does not alter the interpretation of the blood pressure.

### 5.4.13 Blood Pressure Calculations

Blood pressure calculations are made only from the second and third readings. When the form is entered into Datalex, the zero value will be subtracted from the readings to get the actual (corrected) systolic and diastolic blood pressure measurements. All arithmetic will be done by Datalex, although you may hand calculate values for patient care.

If for any reason the observer is unable or has forgotten to complete any portion of the exam, and the participant is gone, leave the items blank on the paper form. If a blood pressure value is missed or forgotten, completely deflate the cuff and start over with a replacement reading after the proper interval. Do not reinflate the blood pressure cuff during a reading. However, under no other circumstances may a replacement reading be obtained. Do not redo a reading that looks unusual to you.

### 5.4.14 Reporting the Blood Pressure Results to the Participant

The patient may wish to know his or her results before the form is entered into Datalex. If so, average the second and third corrected random-zero readings and give the results to the participant. State clearly the systolic and diastolic pressures and offer to write down these values for the participant.

## 5.5 Training Materials

### 5.5.1 Overview of Training Materials

Trainees are first oriented to the subject of blood pressure measurement with a series of lecture-slide presentations. A brief description of each is given here.

1. Blood Pressure Measurement - Problems and Solutions (lecture)

   A general discussion of blood pressure, the history of its measurement, and some of the problems and solutions inherent in its measurement. (pp. 1.3.51.4 - 1.3.51.8)

2. The Random-Zero Device (lecture and slides)

   An explanation of the mechanics and the principles of the operation of this device. (pp 1.3.51.9 - 1.3.51.10)

3. Procedures in Blood Pressure Recording (lecture and slides)

   Step-by-step instructions on how to measure blood pressure using the random-zero sphygmomanometer. (pp. 1.3.51.11 - 1.3.51.15)

4. Local Blood Pressure Equipment Maintenance and Mercury Toxicity Safety Responsibility (notes)

   Step-by-step instructions on how to perform routine maintenance duties on both the random-zero and conventional sphygmomanometers. (pp. 1.3.15.16 - 1.3.51.22)

5. Training Observers in the Clinical Center (lecture)

   Instructions for the Training Supervisor in local training. (pp. 1.3.51.23 - 1.3.51.27)

## 5.5.2 General Plan

After the first three presentations, the trainee may be given a written examination that tests his or her comprehension of the methods used in measuring blood pressure.

Following successful completion of the written examination, the trainee should take a series of blood pressure measurements on live subjects. This exercise will be enhanced by the use of Y-tube stethoscopes because they allow the training supervisor and trainee to listen to the Korotkoff sounds simultaneously and to compare their estimations. Wide discrepancies between supervisor and trainee in the recorded values should be discussed after the record has been completed, in addition to points of technique that may warrant comment.

It should be emphasized that some difference between supervisor and trainee is to be expected, and that exact correspondence should not be expected nor taken even implicitly as a criterion of accurate performance by the trainee. Rather, this step in the certification process is intended to formalize the "live reading," to provide a written record of the results, and to identify gross problems that could be detected only by the supervisor's close involvement with the trainee in this process. Any problems identified by the supervisor or raised by the trainee should be discussed and, as far as possible, resolved.

When the supervisor feels that the trainee has reached a satisfactory level of proficiency in determining the systolic and diastolic blood pressure levels, the trainee should be given The Live Blood Pressure Reading Performance Evaluation. The observer must demonstrate to the training supervisor one or more complete and correct blood pressure determination procedures for 1) cuff selection by correct arm measurement, 2) determination of pulse, 3) determination of peak inflation level using the random-zero or conventional sphygmomanometer, as appropriate and 4) correct blood pressure

measurement following the protocol. The final test to certify an observer will be a videotape test, originally produced by the HDFP specifically for this purpose. The test involves watching a mercury column on a sphygmomanometer and listening to the simultaneous Korotkoff sounds during blood pressure measurements, then recording the systolic and diastolic levels for each on the videotape test sheet. The sheet is then sent to the Data Coordinating Center. The systolic and diastolic readings are entered into a computer and scored.

### 5.5.3 Lecture/Slide Presentations

Five lectures (two with slides) are offered in this section to acquaint the trainee with the subject of blood pressure and its measurement.

The training of potential blood pressure observers should begin with a general discussion of blood pressure and some of the history of blood pressure measurement. The first lecture, "Blood Pressure Measurement - Problems and Solutions," addresses these topics and also reviews some of the problems and solutions in blood pressure measurement. This presentation is quite limited with respect to the physiology of blood pressure regulation and the hemodynamics leading to production of the Korotkoff sounds. The objective instead is to provide sufficient information for <u>any</u> trainee of high school graduate level or beyond, without prior clinical training, to appreciate the significance of the auscultatory signals for blood pressure reading and to recognize those factors of greatest importance for the quality of the readings.

The second lecture, "The Random-Zero Device," is accompanied by a slide series that aids in the explanation of the mechanics and the proper use of this device.

The third lecture, also accompanied by slides, is entitled, "Procedures in Blood Pressure Recording." This presentation gives instructions in the blood pressure measurement technique adopted by the HDFP, SHEP, and now MDRD. Procedures for using both the conventional and the random-zero devices are given.

The fourth and fifth lectures will give the local Training Supervisors a broad overview of the maintenance of the blood pressure equipment with special emphasis on mercury safety and tips/requirements for local blood pressure training.

## 5.5.4 Lecture #1

### Blood Pressure Measurement - Problems and Solutions

What is blood pressure? This question can be answered in many ways - for example, in terms of physiologic and sometimes pathologic processes which contribute to blood pressure regulation. Or, blood pressure can be described in terms of the striking excess in risk of death and disease which accompany high blood pressure levels. For our immediate purposes a more useful and more appropriate answer is, simply: Blood pressure is what is recorded when the measurement methods learned through this training program are carried out.

If we are defining blood pressure in terms of the means of measuring it, the nature of this measurement must be understood. A brief historical sketch is helpful. Measurement of blood pressure by means of the usual mercury manometer, cuff and stethoscope is a method less than 100 years old, although Hales described experimental direct arterial pressure measurements over 200 years ago and Harvey described the circulation of the blood more than 300 years ago.

The start of this century was the period when current, indirect methods were introduced. These were more practical than the lethal method of Hales and qualify as what we would term today a "non-invasive" technique. This indirect method, now almost universally employed, combines the work of Riva-Rocci, an Italian physician who developed the inflatable cuff, and Korotkoff, the Russian physician who described his auscultatory findings, heard through a stethoscope placed over the brachial artery, as an improvement over mere palpation of the radial pulse, a technique limited to detecting systolic pressure alone.

The report of Korotkoff's first observations is an informative summary of the specific sounds he described:

On the basis of his observations, the speaker has come to the conclusion that the completely compressed artery under normal circumstances does not produce any sounds. Utilizing this phenomenon, he proposes the auditory method of determining the blood pressure in man. The cuff of Riva-Rocci is placed on the middle third of the upper arm, the pressure within the cuff is quickly raised up to the complete cessation of circulation below the cuff. Then, letting the mercury of the manometer fall, one listens to the artery just below the cuff with a children's stethoscope. At first, no sounds are heard. With the falling of the mercury in the manometer, down to a certain height, the first short tones appear; their appearance indicates the passage of part of the pulse wave under the cuff. It follows that the manometric figure at which the first tone appears corresponds to the maximal pressure. With the further fall of the mercury in the manometer, the systolic compression murmurs are heard, which pass again into tones (second). Finally, all sounds disappear. The time of the cessation of sounds indicates the free passage of the pulse wave; in other words, at the moment of the disappearance of the sounds, the minimal blood pressure within the artery preponderates over the pressure in the cuff. Consequently, the manometric figures at this time correspond to the minimal blood pressure. Experiments on animals gave confirmative results. The first sound-tones appear (10 to 12 mm) earlier than the pulse, for the palpation of which (e.g., in the radial artery) the inrush of the greater part of the pulse wave is required. [Quoted from Ruskin, A. Classics in Arterial Hypertension, Charles C. Thomas, Springfield, 1956 (pp. 127-128).]

With further refinement in criteria by which changes in sound quality are to be judged, we arrive very nearly, but not quite, at the level of technological advance applicable to the conventional mercury sphygmomanometer today. In summary then, we may define blood pressure as the phenomenon measured when the cuff, mercury manometer and

stethoscope are used in the standard manner by a trained observer to assess the cardiovascular status of a subject.

Discussion of blood pressure in these terms would be seriously incomplete, however, if we did not take account of the fact that important problems of measurement exist. It is imperative that these problems be recognized and, as far as possible, overcome. What are they?

An excellent review by Evans and Rose[7] distinguishes first random variation within each subject, and second, systematic variation which they subclassify as follows: "(i) alarmingly large differences in estimation between observers, sometimes as large as 15 mm Hg..., (ii) effects of the circumstances of measurement, both emotional and physical (especially recent physical activity or change of position), (iii) seasonal changes, and (iv) relatively small errors due to overestimation of pressures in fat arms...."

If these are the major categories of problems, what can be done to deal with them? With respect to random individual variation for each person, we obtain multiple readings on each occasion of observation and use as our estimate of blood pressure an average of two readings, always excluding the first inflation of the cuff (used only to estimate the peak inflation level).

What about the systematic biases? Taking those listed in reverse order, we may say the following. The fat arm should be wrapped in a cuff of appropriate size - either the large arm cuff, or if necessary, the thigh cuff - to exclude the effect of a single cuff size in giving falsely high readings for participants with excessive arm girth. Effects of circumstances, especially activity and posture, can be dealt with by requiring that all readings be taken in the sitting position, only after a minimum period of 5 minutes seated at rest, according to carefully prescribed procedures. As to differences

between observers, a systematic difference as large as 15 mm Hg would indeed be alarming, and in fact unacceptable. In still another publication dealing with measurement of blood pressure, Rose presented in greater detail some components of the remaining major problem, observer differences in blood pressure readings. These components are considered as of two types, one type affecting chiefly the mean of a series of measurements, the other type chiefly distorting the reported frequency distribution of readings. This latter type includes terminal digit preference, which is the unconscious tendency to choose one digit over others in assigning the value of a reading and the prejudice against certain values. Factors affecting mean differences between observers include mental concentration or reaction time, hearing acuity, confusion of auditory or visual cues, interpretation of sounds, rates of inflation and deflation of the cuff, and reading of the moving column of mercury.

Are there answers to these problems? Regarding hearing acuity, deficiencies can be excluded by satisfactory performance on the videotape test. Regarding the effects of prejudicial reading, a device can be used that is designed primarily to overcome this tendency, the Random-Zero device. For all the remaining problems, we have a single answer: TRAINING. We will talk shortly about the random-zero device and about the standard procedures to control the circumstances of measurement. Training will occupy the rest of our attention to blood pressure measurement, for a good number of hours. The method of training and its specific objectives are therefore worth brief discussion now.

Training in blood pressure measurement will take three forms. First, there will be lecture and slide presentations to acquaint you with the proper procedures for measuring blood pressure and also to familiarize you with the random-zero device. Second, you will take actual live blood pressure readings. The objective of live reading practice is to become thoroughly familiar with the details of standard procedure so

that their performance becomes a matter of habit. Proficiency in this aspect of training will be assessed under observation by the training supervisor. And third, your ability to measure blood pressure accurately as a result of this training will be tested using a videotape to simulate the fall of mercury with accompanying Korotkoff sounds during an actual blood pressure measurement. You will be required to determine the systolic and diastolic levels for each subject in the film, within predetermined limits.

Our responsibility, in supervision of this training program, is to offer all possible assistance to each of you, individually, in meeting these requirements and in completing each step necessary for your certification as a qualified blood pressure observer. We trust that you will take every opportunity to raise questions and indicate to us any problems you may have in working with these materials and completing the program satisfactorily. Accurate blood pressure measurement is critical, and there are methods available to substantially reduce the systematic errors that we have recognized. Your participation in this program will take advantage of these methods to assure a highly qualified group of observers.

### 5.5.5 Lecture #2

The Random-Zero Device

The random-zero device is essentially a mercury sphygmomanometer like the conventional device in common use. It differs in the important respect that a mechanical addition allows the mercury level in the column to be varied for each reading and concealed from the observer until the systolic and diastolic readings have been completed. This arrangement thus avoids the observer bias which is often at play when the observer knows the actual pressure level as the reading is carried out.

How this device is operated and how its mechanical features fulfill the objectives of its design can best be appreciated by inspecting the device, by practicing its use, and by preliminary inside view. We will take this preliminary view first, through a series of slides, and later practice with it.

### 5.5.6 Slide

Number Script For Slide

1. As we have already discussed, the random-zero device and the conventional mercury sphygmomanometer are essentially very similar. This can be seen in comparing the two devices side by side. The random-zero device <u>is</u> unique, however, as the following slides will show.
2. The crucial distinction is the wheel on the right-hand side of the random-zero casing. To get a little closer to the workings of the device, we may remove the front of the casing, to find
3. the manometer column, the cuff and its connections, and one notable feature: a lever controlling the reservoir outlet. This lever is always closed for carrying the device (i.e., turned to the left) and opened (i.e., turned to the right) for operating it. You might notice also that the mercury rests at a level well above 0 mm, even though the cuff is not inflated. Let's take a close look at the mechanism that accomplishes this to see how simple it really is.

4. To remove the rear portion of the casing (which should be done only by the Training Supervisor or other authorized staff member, and only when necessary for adjustment or standardization) one needs only to remove two screws from the upper face of the device, and two from the lower rear.
5. Now we can get a better look at the inside. You will notice right away that the wheel you spin from outside is larger in diameter than you might have guessed, and it occupies a central position in the internal mechanism of the device. The movable rear wall of the chamber is the large round disc up above, which is ringed with its rubber seal.
6. From directly behind you can see the wheel in relation to the chamber wall, and also the black rubber air hose connecting the cuff with the top of the mercury-filled plastic hose which connects the bottom of the reservoir with the chamber.
7. In this view you can see the control knob which the observer operates to open and close the connection between chamber and reservoir. Also, nearly the whole movable chamber wall can be seen. What gets in the way is a small aluminum cylinder cam which we will want to focus on in a moment. From the side we can see the three key elements that give this device its special value: the rubber-edged wheel which is spun (from the outside) before each reading; the cylindrical aluminum cam which contacts the rubber rim of the wheel and spins at the same time (and its bevelled forward end which extends forward in varying degrees depending where it comes to rest); and finally the movable rear wall of the chamber, which will be arrested in its backward movement when pressure is applied as soon as it contacts the cam. When the cuff is inflated, pressure on the reservoir will force mercury into the chamber until the wall reaches the cam and stops. The amount of mercury in the chamber at this point will determine the "zero" reading for this one time, aiding the observer to make objective readings unaffected by knowledge of the true reading.

5.5.7 Lecture #3

Procedures In Blood Pressure Recording

These procedures for blood pressure recording were developed after extensive consideration and discussion of numerous approaches to measurement techniques. In addition to the selection of instruments and specification of criteria for measurement, we specify methods for the entire sequence of steps in blood pressure recording. For all observers, whether inexperienced in blood pressure measurement or accustomed to different procedures, it will be important to become intimately familiar with these procedures and to carry them out, as early as possible, as a matter of habit. As an introduction, the following series of slides is presented to demonstrate the steps involved for the recording of blood pressure. The sequence presented here illustrates use of both the random-zero and the conventional sphygmomanometers.

5.5.8 Slide

Number Script For Slide

Equipment and Supplies

1. The equipment needed by each observer includes a random-zero sphygmomanometer in good condition,
2. or a conventional sphygmomanometer (for optional use during MDRD screening)
3. Access is needed to the full set of cuff sizes for this population. These are commonly referred to as the child (or pediatric) or small adult, adult (or regular), large and thigh (or extra large) cuffs, respectively.
4. The inflation bulb should operate smoothly and should perhaps be individualized to each observer.
5. The stethoscope, in good condition, should be switched for use of the bell in listening to the Korotkoff sounds.
6. A watch with a sweep second hand or with a digital second display, or a stop watch, is needed for measurement of the pulse rate and for timing certain other steps until they become a matter of habit.

7. A measuring tape in metric units is required for determination of the correct cuff size for each participant.
8. A ball point pen should be used for all data recording, preferably with medium or larger point, and black ink.
9. Requirements for furniture are simple but must provide for a comfortable resting position of the arm with mid-cuff at heart level.
— A Mayo stand (or other similar device) should be available for use in the annual standing blood pressures. (Note: No Slide.)
10. The appropriate study form must be in place before measurement begins.

### 5.5.9 Arm Measurement

11. Measurement of the arm is required for selection of the proper cuff. For this measurement, the arm should be bare.
12. The measurements are taken on the right arm, with the participant standing, holding the forearm horizontal.
13. Arm length is measured from the acromion or bony extremity of the shoulder girdle,
14. to the olecranon, or tip of the elbow.
15. The full arm length from acromion to olecranon is measured, and
16. the midpoint is marked on the dorsal surface of the arm.
17. With the participant's arm relaxed at the side, the arm circumference is measured by drawing the tape snugly (without indenting the skin) around the arm at the level of the midpoint marking. Care must be taken to keep the tape horizontal.
18. The chart of arm circumference measurements and corresponding cuff sizes is consulted, and
19. the proper cuff size is checked. Indicate this cuff size on the form.

### 5.5.10 Preparation for Actual Readings

20. The participant should then be seated with the elbow and forearm resting comfortably on a table with the palm of the hand turned upward. The area to which the cuff is to be applied must be bare.

21. The brachial artery is located by palpation and marked,
22. as is the midpoint of the rubber bladder within the cuff. Often this point is marked on the cuff itself.
23. The cuff is then wrapped about the arm so that the midpoint of the bladder lies over the brachial artery, and the mid-height of the cuff is at heart level.
24. The random-zero sphygmomanometer is then connected with the cuff.
25. The manometer is positioned so that the midpoint of the column is at the observer's eye level when in position to carry out the measurement of blood pressure.
26. The radial pulse is located, and
27. with the valve in the OPEN position, the cuff is inflated quickly to 200 mm Hg.
28. The pressure is maintained at 200 mm Hg for 5 seconds (a slow count to 5) and the valve then turned to the CLOSE position. A pulse measurement is taken. If the pulse is still detected, the cuff is inflated slowly until the pulse disappears. Either the first or the second of these procedures identifies the Observed Pulse Obliteration Pressure.
29. The cuff is quickly and completely deflated.
30. The observed value and the "zero" value are used to calculate (by subtraction) the Corrected Pulse Obliteration Pressure. All three are recorded on the form.
31. The sum of the "maximum zero" level for this Random-Zero (found next to mercury column) plus 20 mm Hg plus the Corrected Obliteration Pressure equals the Peak Inflation Level.

### 5.5.11 Pulse

32. After the period of 5 minutes at rest has been completed, the radial pulse is counted for a timed interval of exactly 30 seconds.
33. The 30-second count is recorded and multiplied by 2 to give the full number of beats per minute.

### 5.5.12 First Blood Pressure Reading

34. To perform the measurement of blood pressure itself, the brachial artery is again palpated. Note that the arm remains bare.
35. The wheel of the random-zero is gently spun several times with the valve in the OPEN position.
36. The stethoscope earpieces are put in place with the earpieces positioned forward, and
37. the bell of the stethoscope is placed carefully and without excessive pressure over the brachial artery, just between the elbow crease and lower edge of the cuff.
38. With the valve still in the OPEN position, the cuff is inflated quickly and smoothly to the peak inflation level or to 200 mm Hg, whichever is higher.
39. By closure of the thumb valve, this pressure level is maintained for 5 seconds (count to 5 slowly).
40. The valve is then turned to the CLOSE position.
41. The cuff is then deflated very steadily at 2 mm Hg per second,
42. to a level 10 mm Hg lower than the level of the last Korotkoff sound heard.
43. The mercury level is now dropped quickly to the "zero" level for this reading.
44. The cuff is then disconnected and the stethoscope removed.
45. The observed values for the SBP, DBP, and "zero" values are recorded.
46. The "zero" value is subtracted to give the corrected SBP and DBP. This completes the first actual reading.

### 5.5.13 Between Readings

47. If the cuff is uncomfortable for the participant you may remove it, and
48. the observer will raise the participant's arm overhead for 15 seconds without the participant's assistance.
49. The arm is then lowered gently,
50. if the cuff was removed it should be replaced, and
51. the random-zero sphygmomanometer is reconnected.

### 5.5.14 Second Blood Pressure Reading

52. The second reading is carried out exactly as the first, following several gentle spins of the wheel on the random-zero with the valve in the OPEN position.

53. The observed SBP, DBP, and "zero" values are recorded,

54. The corrected values are calculated by subtraction of the "zero" value.

### 5.5.15 Between Readings/Third Blood Pressure Reading

--- The cuff may be removed once again and the entire sequence is repeated from having the observer raise the participant's arm overhead for 15 seconds to taking a third Blood Pressure Reading. (Note: No Slide.)

--- As before, the observed SBP, DBP, and "zero" values are recorded. (Note: No Slide.)

--- The MAP (Mean Arterial Pressure) for the visit will be the average of the second and third MAPS. (Note: No Slide.) This will be calculated by Datalex. The blood pressure observer may supply the participant with the corrected blood pressure values if requested.

## 5.5.16 Lecture #4

## Local Blood Pressure Equipment Maintenance And Mercury Toxicity Safety Responsibility

The condition of the instruments for blood pressure measurement is too often ignored in common practice and should be a special responsibility of the blood pressure observer. This person should be acquainted with mercury toxicity safety procedures as well as construction and function of all the blood pressure equipment. The cuffs and stethoscope, cleanliness and general working order can usually be determined by simple inspection. For either the conventional or random-zero sphygmomanometer, handling of breakable parts and of mercury and oxidized waste requires more careful attention. Guidelines for suggested maintenance procedures for the manometers are outlined here.

### 5.5.17 General Guidelines

1. The objective of maintenance of all sphygmomanometers is to ensure their accuracy for blood pressure measurement. The manometer column must be clean and the system free of mercury leakage. The zero level for the conventional device should be accurately read as 0 mm Hg at the top of the mercury meniscus. The "zero" levels for the random-zero device should have a range of approximately 20 mm Hg between the maximum and the minimum "zero" levels, the absolute values being 4 mm Hg or more for the minimum "zero" level and 30 mm Hg or less for the maximum "zero" level. These values should remain constant for a given instrument, and the maximum "zero" for each instrument should be indicated by a label on the front of the machine itself for use in the calculation of peak inflation levels for each reading taken with the device.

2. These devices should be cleaned and checked thoroughly on a quarterly basis (approximately every three months). More frequent inspections should be made to ensure there has been no mercury spillage or leakage

and no obvious malfunction of the device. Instruments used in clinics should be inspected weekly. Those inspections should include a check of zero levels, mercury leakage, operation of valves, manometer columns for dirt or mercury oxide deposit, and condition of all tubing and fittings.

3. Procedures for inspecting the random-zero manometer are outlined below in Section 5.5.19. The manometer portions of both instruments are produced by W. A. Baum Company (Copiague, New York 11726) so that maintenance for this portion of the two devices is the same, as is the case for cuffs, bulbs, and air control valves. More detailed instructions covering these parts are provided in the Baumanometer Service Manual which is available from the W. A. Baum Company.

5.5.18 Common Problems with — and Solutions for — the Manometer

1. Problem: Dirty manometer column.
   Solution:
   a. This is due to dirty or oxidized mercury and is usually evident near the zero. Oxide and dirt near random-zero machines "zeros" can result in too high "zero" readings because mercury sticks on the column wall above its equilibrium level. This does not affect conventional manometer readings, but it is hard to see the meniscus, and hence to check the actual zero.
   b. Remove the glass manometer column. See Baum instructions for removal of column from conventional manometers.
   c. Clean the glass column from its top towards its zero, with the "super" pipe cleaners available from Baum. Hold the column over a container to catch mercury as the cleaner is pushed through and brush the soiled end of the cleaner into the container.

2. Problem: Leaked mercury.

   Solution:

   This can be due to any of the following:

   a. Loose or leaky screw cap at top of manometer
   b. Manometer column cracked or chipped, or improperly seated
   c. Leaky manometer column gaskets
   d. Tilting the random-zero manometer with the mercury reservoir valve OPEN.
   e. Loose or leaking random-zero manometer bellows on bleed screw cap.

3. Problem: The mercury level will not remain constant when the bulb valve is closed.

   Solution:

   a. Connect the manometer to a cuff which is around a one pound coffee can. Pump up the cuff and begin to pinch the tubing closed, starting at the manometer tubing.
   b. By a process of pinching the tubing at 1 to 2 inch intervals up to the cuff and then down to the bulb, you will locate an air leak.
   c. If an air leak is found in the cuff bladder or in the tubing other than the connections, the bladder may need to be replaced.
   d. If the air leak is found in the connections or in the bulb valve, a little silicone spray may alleviate the problem.

### 5.5.19 Inspection of the Random-Zero Manometer

Unless obviously damaged due to dropping or other accident, the random-zero sphygmomanometer is expected to operate without disturbance of its measurement performance. Periodic checking should be done, however, to ensure against undetectable internal leakage or malfunction of the "randomizing" mechanism.

1. Place device in usual operating position, with reservoir valve OPEN (to side).

2. Remove mounting screws from the front and rear of the wooden or plastic casing and remove the casing keeping the instrument upright at all times.
3. Inspect the base and moving parts for any evidence of mercury leakage.
4. Bleed the air out of the system and check for mercury leaks. Using a 30 ml or larger syringe and a length of tubing, apply greater than 200 mm Hg pressure to the mercury column. (A syringe gives faster and better control than a cuff and a bulb for this purpose, but the observer must be careful not to pull negative pressure. If a cuff is used, it can be wrapped around a one pound coffee can.) Watch the rise of mercury in the chamber and maintain or increase the pressure until the mercury rises into the narrow vertical stem at the top of the chamber. If mercury does not enter the stem despite prolonged high pressure, deflate the cuff and repeat, after slightly opening the thumbscrew at the top of the stem. This will permit escape of any trapped air. When the mercury has entered the stem, close the thumbscrew firmly (but not excessively tight), and deflate the cuff.
5. Verify the maximum "zero" obtainable.
   a. The bellows valve should be in the OPEN position and no pressure should be in the cuff. The cam should rotate freely.
   b. Set the cam manually in such a position that the level on the end of the cam will contact the moving wall of the chamber after the shortest possible displacement of this wall toward the cam. This position draws the least mercury into the reservoir and produces the highest "zero" level for the amount of mercury in the device at this time.
   c. Inflate the cuff above 200 mm Hg and maintain it at this pressure until the chamber wall has come to rest against the bevel of the cam.
   d. Turn the valve to CLOSE, wait a full 5 seconds, and deflate and disconnect the cuff.
   e. Record the zero level. It should compare closely (within 2 mm Hg) with the value on the label on the face of the manometer.

6. Verify the minimum "zero" level obtainable.

   a. Repeat exactly as for (5) above, except to set the cam so that the moving wall of the reservoir will move its maximum distance before contacting the cam. This position draws the most mercury into the reservoir and produces the lowest "zero" level for the amount of mercury in the device at this time.

   b. Ensure that full pressure in the cuff is maintained until the wall of the chamber comes to rest against the bevel of the cam. This may take several seconds.

   c. Turn the bellows valve to CLOSE and deflate and disconnect the cuff.

   d. Record this "zero" level. It should compare closely (within 2 mm Hg) with the value determined when the machine was calibrated.

7. Adjustment of zero levels.

   Changes of zero levels are due either to loss of mercury or to air leakage at the bellows air bleed screw. Accuracy of readings is not affected. To adjust zero levels, however, mercury must be added to or removed from the system.

   CAUTION: Mercury vapor is very toxic. Tiny droplets vaporize more rapidly than bulk. All loose mercury must be collected and inactivated. One effective and convenient product for mercury vapor reduction is "HgX", a powder produced by Acton Associates, 1180 Raymond Blvd., Newark, NJ 07102. It is recommended that all work be done in a container such as a plastic dish pan when mercury is to be transferred.

   a. If the zero levels are too low:

      (1) Open the bellows control valve and the valve at the top of the mercury reservoir, unscrew and remove the knurled cap at the top of the manometer column, and remove the air bleed screw at the top of the bellows chamber.

      (2) Pour clean mercury into the top of the manometer tube, using a hypodermic syringe barrel or tight paper cone as a funnel. (As Baum writes, mercury can be cleaned of floating dirt and

oxides by pouring it through a rolled cone of ordinary scratch paper with pinhole at its apex. Note that some mercury will stick on and in the paper, so handle with care). About 400 grams (or 14 ounces) of mercury are needed to fill an instrument for a zero range of near 10 to 30 mm.

(3) Firmly screw the knurled cap onto the top of the manometer column and apply pressure to the mercury reservoir until the mercury rises into the vertical air column at the top of the bellows chamber. Tighten the air bleed screw quickly and firmly while the mercury is a short distance into the vertical air column.

(4) Apply enough additional pressure to raise the mercury to near the top of the manometer column if it is not already that high. Then release the pressure, thus to collect mercury droplets and clear the column of air bubbles. There are likely to be air bubbles trapped on the walls of the plastic tube at the bottom rear. These can sometimes be removed by tapping the tube sharply, but they are, at any rate, of no consequence.

(5) Determine zero range and adjust as needed (see above).

b. If the zero levels are <u>too high</u>: Unscrew and remove the knurled cap from the top of the manometer column. Using a syringe with a small tube, such as a catheter, remove the mercury from the manometer. (Or, if these are unavailable, pour surplus mercury from the open manometer column. See Baum instructions. Be sure that the mercury reservoir valve is closed before inverting the manometer to pour the mercury out.)

8. <u>Check whether the spin wheel and cam spin freely</u>.

a. Turn the bellows valve on the front of the manometer to OPEN and allow the wall of the chamber to move back to its resting position.

b. Spin several times the rubber-rimmed wheel used in setting the "zero" level for each reading. Note whether the cam spins freely and whether it is excessively loose.

c. Adjust the spin by slightly loosening or tightening the mounting screw at the end of the cam.

d. After any such adjustment, recheck the spinning wheel repeatedly to ensure against excessive tightness or looseness of the cam. If spin wheel and cam are stuck (with bellows control cock open and all pressure released) or the rise of the mercury column is jerky as pressure is raised, there is usually binding or friction between the bellows plate center boss and the centering pin. Accuracy of readings has not been affected. A drop of good, light machine oil takes care of most such problems.

9. To remove the manometer column for cleaning or for inspection of it and of gaskets:

a. Set the cylindrical cam for maximum bellows volume and open the bellows control valve.

b. Raise the reservoir pressure to about 280.

c. Close the bellows valve and release pressure on the reservoir.

d. Tilt the sphygmomanometer to the right (reservoir on down side) until all mercury has disappeared below the manometer column. Close the reservoir valve (handle to front). Rest the device on its right side with the spin wheel above the table surface.

e. The manometer column may now be removed.

10. Maintenance requirements are minimal, but essential.

a. A very occasional drop of light machine oil is recommended on moving parts including the bellows plate centering pin.

b. Do not, however, oil either the bellows control valve stem or the mercury reservoir valve.

c. Ensure that moving parts are free without too much slack.

## 5.5.20 Lecture #5

### Training Observers In The Clinical Center

There are three distinct sections involved in the responsibility of the local Training Supervisors. First is the preparation for the training session. Second is the time scheduling of the sessions. And third is the documentation of certification to the Data Coordinating Center.

### 5.5.21 Preparation for Training Observers

A. Gather all the blood pressure equipment.
   1. Both the conventional and random-zero manometers
   2. All four basic sizes of blood pressure cuffs with bulbs
   3. A bell stethoscope

   Familiarize yourself with all the blood pressure equipment. Prepare for mercury safety procedures and prepare an equipment maintenance schedule. Check all random-zero sphygmomanometers for maximum and minimum zero levels. The standard sphygmomanometers should be checked so that the top of the mercury meniscus is at the zero marking. The stethoscopes should be clean and turned to the bell. The cuffs and air valve should be checked for air leaks.

B. Gather all the Training Materials.
   1. This training manual
   2. The appropriate forms and paper
   3. 2 X 2 slide projector and carousel
   4. videotape machine
   5. Black ball-point pen

   You should carefully familiarize yourself with all the training materials. Only you know how much practice will be needed for you to present the lectures to your trainees. Be sure you have plenty of photocopies of all the forms (the Written Examination, the Live Blood Pressure Performance Evaluation Sheet, and the Videotape Test Sheet). Familiarize yourself with the operation of the slide projector and videotape machine.

### 5.5.22 Training Tips

A. Schedule the training sessions over a period of days. An unhurried schedule gives the trainee a chance to absorb and demonstrate the procedures and knowledge with more confidence. Remember, you may be training someone who needs to unlearn previously learned blood pressure procedures. Also remember the stethoscope can cause ear discomfort when used for several hours at one time.

B. Try to keep the group size workable. The lectures may work for a large group, but consider the waiting/noise factor when scheduling the written test, blood pressure practice/evaluation and the videotape viewing.

C. The certification of the trainee and duties as an observer should not be planned for the same day. The trainee cannot complete the certification and begin taking participant blood pressures that same day. Plan time to allow for the return of all the documentation to the Data Coordinating Center, and return of the notice of certification. If scheduling requires, it may be possible to confirm certification of observers by telephone once all materials have been received. We realize that infrequently a crisis will arise. The videotape test values may be called in by telephone and scored that day, with the written documentation following in the mail, but this should be a rare occasion.

### 5.5.23 Documentation of Certification

A. Each person in the Clinical Center that will be filling out any part of a blood pressure form will need a study ID code. This includes the blood pressure observers. Only one code number should ever be assigned one person, no matter how many changes in status might occur.

B. The Written Examination should be taken by the trainee and graded by the supervisor. If there are any differences in responses, it should

be discussed and clarified. The supervisor should indicate those responses that were discussed by initialing them.

C. The Live Blood Pressure Reading Performance Evaluation should be carefully followed to ascertain that the trainee has a clear understanding of the procedures. This evaluation should be completed by the supervisor as a passive observer. Avoid prompting the trainee. The trainee should complete one or more complete and uninterrupted exercises of the full procedure. Errors of procedure should be reviewed, discussed and corrected. When carried out without procedural errors, this record should be completed, signed and included with the certification packet of the trainee.

D. The practice videotape should be employed to familiarize the trainee with the process of the videotape. Do not overexpose the trainee to the actual videotape test. When the videotape test is taken, remind the trainee to insert leading 0's where necessary and to complete the entire form. The test will be graded upon arrival at the Data Coordinating Center. If a systematic problem is discovered via computer scoring, the Data Coordinating Center will instruct you as to the type of problem discovered. The specific problem should not be identified to the trainee, as this may artificially bias the trainee's responses. Retraining, possibly by Y-tube readings, may help to identify and correct the problem. If the problem is not corrected within several retrainings, the problem is probably auditory and the trainee would need to be excluded from taking blood pressures. The Data Coordinating Center will need to have complete documentation of the certification before the trainee can be employed as a blood pressure observer. We suggest the supervisor keep the originals and send photocopies to the Coordinating Center. The Coordinating Center will instruct the Training Supervisor when recertifications should be scheduled, on an annual basis in the Spring.

## 5.6 Certification Procedures And Criteria

### 5.6.1 Three Steps Needed For Certification

In order to standardize the previously described methods of blood pressure measurement and to ensure that a high level of performance is attained, a three part training session has been developed. After successful completion, an observer is certified to take blood pressures in the study program. The three steps needed for certification are enumerated below.

1. The first step of blood pressure training is the completion of the Written Examination after lectures 1 - 3 have been presented. This is a short examination consisting of questions that test the blood pressure observer's knowledge and understanding of the measurement technique detailed in the training course.
2. The second step is the successful completion of The Live Blood Pressure Reading Performance Evaluation. The training supervisor is to verify the correct procedure for blood pressure measurement by observing the trainee in one or more complete and uninterrupted exercises of the full procedure. When carried out without procedural errors, this record should be completed, signed and included with the certification packet for the trainee. Errors of procedure should be reviewed, discussed and corrected, until one completed determination is accomplished without error.
3. The third step is a series of blood pressure readings presented on a videotape to test the observer's identification of the systolic and diastolic Korotkoff sounds. This tape mimics the actual blood pressure measurement setting by providing a series of blood pressure readings which consist of both the visible falling of the mercury in a sphygmomanometer and the audible Korotkoff sounds. An observer is certified if the criteria of the scoring procedure are successfully met. The criteria of the scoring procedure are not available to the

Clinical Center or to the observers. The scoring will be done via computer at the Data Coordinating Center upon receipt of observer's test sheets.

As a means of maintaining a high level of quality and standardization over time, blood pressure observers will be recertified annually. This recertification will involve, at a minimum, repeated testing by viewing the videotape and submitting a completed test sheet, as well as live measurement performance evaluation. The Data Coordinating Center will notify the Clinical Centers as to the schedule and requirements of the recertification. A further description is in Section 5.7.2.

### 5.6.2 Instructions for Taking the Videotest

Viewing of the videotape, "Measuring Blood Pressure," may be done in a group or individually. The videotape consists of practice readings followed by twelve systolic and diastolic sequences. After each sequence, the observer should record, on the recording sheet provided, the systolic and diastolic reading for that sequence. All entries should be completely legible and written in black ink. Leading 0's should be entered if appropriate. The manometer in the videotape is read exactly as one would be read in actual practice. Each blood pressure should be read to the nearest even digit.

### 5.6.3 Three Study Forms Required For Certification Procedures

The three study forms required for certification include:

1. The Written Examination (and its key);
2. The Evaluation Sheet for <u>The Live Blood Pressure Reading Performance Evaluation</u>; and
3. The Videotape Test Sheet.

These three forms may be found under separate cover.

## 5.7 Blood Pressure Measurement Quality Control

### 5.7.1 Overview

There are two primary methods for monitoring the performance of trained observers in the measurement of blood pressures during the course of a clinical trial. The first is the completion of an Annual Recertification set of procedures. The second is the three times per year monitoring by the Data Coordinating Center of all observers for digit preference.

In addition to these, MDRD has adopted and instituted a comprehensive program to insure the collection of high quality blood pressure measurements. Factors contributing to this include:

1. Recruitment of the most qualified personnel.
2. Standardized training and certification.
3. Retraining of observers having difficulties with standardized measurements.
4. Bimonthly (every other month) observations by the Training Supervisors of data collection techniques of the Blood Pressure Observers on either a patient or MDRD personnel, using the checklist at the end of this chapter. One checklist is used for each blood pressure observer. These should be kept on file and will be reviewed at site visits.
5. Bimonthly (every other month) simultaneous Y-Tube observations of each Observer by the blood pressure Training Supervisor on either a patient or MDRD personnel (described in Section 5.7.4).
6. Frequent staff meetings to provide feedback.
7. Continuous editing and analysis of data by the Data Coordinating Center.
8. Presentation of data analyses to the Clinical Centers by the Data Coordinating Center to provide feedback three times per year.
9. Equipment maintenance program (described in Section 5.5.16, Lecture #4).
10. Documentation of the Bimonthly Checklist, Y-tube stethoscope observations, and the dates of the Random-Zero Sphygmomanometer weekly inspections will be sent to the DCC on a quarterly (every 3 months) basis.

### 5.7.2 Annual Recertification and Retraining

As with the initial certification process this recertification process includes the successful completion of:

* a written test
* a live evaluation
* a videotape test.

Training Supervisors will be retrained centrally every spring. Recertifications for the other Blood Pressure Observers will also be annual, but after the recertification of the Supervisors (unless an Observer is centrally recertified).

The recertification procedures for the Blood Pressure Observers will be conducted at the Clinical Centers. However, scoring of the video tests will be done by the Data Coordinating Center, WHICH IS RESPONSIBLE FOR IDENTIFYING WHO IS A CERTIFIED BLOOD PRESSURE OBSERVER. Results of the recertification tests will be telephoned (and subsequently mailed) to a Clinical Center within three (3) working days of receipt of the test data from the clinic. A report based upon the results of these tests may be presented to the Steering Committee and the Policy Board. This report would describe how well the observers are measuring blood pressure levels under standardized conditions, and how many observers had difficulty being recertified.

Of course, the results of the tests may indicate that an observer may need to be retrained in some or all aspects of blood pressure measurement. If this is required, this person will discontinue the measurement of blood pressure levels for the trial until he or she is successfully recertified by the Coordinating Center. Central retraining may be required.

Also, if an observer misses a recertification cycle, he or she must repeat the training program.

### 5.7.3 Monitoring for Digit Preference

It is well documented in other large blood pressure studies that even well trained observers have the capability to lapse into an unconscious digit preference over time. Digit preference is defined as a predilection to record the terminal digit of a blood pressure measurement as either a "0", or a "2", or a "4", or a "6", or a "8", rather than the actual value. For example, an observer with a "0" digit preference may record an 82 mm Hg DPB (or a 78 mm Hg) as 80 mm Hg.

**NO OBSERVER SHOULD EVER HAVE A DIGIT PREFERENCE.**

The recertification process should dampen, on an annual basis, any incipient digit preference, but three times per year monitoring and presentation of actual blood pressure measurements by the Data Coordinating Center will identify problems more immediately. If a problem is identified, the blood pressure consultant to the MDRD (or his designee) will be notified and corrective procedures implemented. Possible re-training and recertification may be necessary before the regularly scheduled certification.

### 5.7.4 Bimonthly Y-Tube Stethoscope Observations

Y-Tube stethoscope observations are made in conjunction with the initial training and for bimonthly quality control. The Training Supervisor has the Observer go through the entire blood pressure measurement procedure using a quality control checklist. The Observer and Supervisor listen with the Y-tube and record the values on separate sheets. Two measurements on one subject are obtained and will be kept on file and reviewed at site visits.

It should be emphasized again that some difference between supervisor and trainee is to be expected, and that exact correspondence should <u>not</u> be expected nor taken even implicitly as a criterion of accurate performance by the trainee. Rather, this process is intended to formalize the

"live reading," to provide a written record of the results, and to identify gross problems that could be detected only by the Supervisor's close involvement with the Blood Pressure Observer. Any problems identified by the Supervisor or raised by the Observer should be discussed and, as far as possible, resolved.

### 5.7.5 Responsibilities of the Data Coordinating Center and The Training Supervisors

It is the responsibility of the Data Coordinating Center to centrally train and certify the Training Supervisors. Whereas it is primarily the responsibility of the Training Supervisors to return to the Clinical Centers and train other observers, these other observers may also be trained centrally by the Data Coordinating Center. However, only the Data Coordinating Center is able to certify an observer, as described above.

If, between recertifications, the Data Coordinating Center and/or a Training Supervisor have evidence that an observer is not performing well, the three parties will meet to discuss the matter. It may be necessary for the Data Coordinating Center to temporarily rescind a certification and retrain the observer. In this case, until the observer is recertified, he or she may not take blood pressure measurements for MDRD.

It is also the responsibility of the Data Coordinating Center to monitor the specific activities of the Training Supervisors. In addition to the continuous monitoring of all incoming blood pressure data (eg., for digit preference or bad values), the files of the bimonthly blood pressure checklists and Y-tube observations will be reviewed at each site visit for completeness and accuracy. Also at these site visits, the Training Supervisors themselves will undergo checklist monitoring and Y-tube observation. Finally, the Training Supervisors themselves will be recertified centrally every Spring, before the annual recertification of the other Blood Pressure Observers.

## 5.7.6 Bimonthly Checklist for Monitoring MDRD Blood Pressure Observers

(To be kept on file at the Clinical Center)

Performing MDRD Technician Certification Code #_____

Observer MDRD Technician Certification Code #_____

Date Observed ____/____/____ (Month/Day/Year)

---

Instructions: Check if procedure step is carried out correctly.

| | | Procedure | Comments |
|---|---|---|---|
| 1. | ____ | Measures arm for correct cuff size | _____ |
| 2. | ____ | Palpates brachial artery | _____ |
| 3. | ____ | Marks brachial artery point | _____ |
| 4. | ____ | Check center of bladder and wrap cuff correctly | _____ |
| 5. | ____ | Wraps cuff center of bladder over brachial pulse | _____ |
| 6. | ____ | Calculate peak inflation | _____ |
| 6a. | ____ | Finds pulse obliteration point using R-Z manometer | _____ |
| 6b. | ____ | Calculates peak inflation using R-Z manometer | _____ |
| 7. | ____ | Leaves subject for 5 min. rest, instructs on posture, smoking, talking | _____ |
| 8. | ____ | Takes radial pulse | _____ |
| 9. | ____ | Opens bellows valve, waits for mercury to settle | _____ |
| 10. | ____ | Turns thumb wheel gently | _____ |
| 11. | ____ | Places stethoscope in ears | _____ |
| 12. | ____ | Palpate brachial artery, position's bell of stethoscope on brachial artery | _____ |
| 13. | ____ | Inflates rapidly to R-Z peak | _____ |
| 14. | ____ | Count full 5 seconds with pressure steady | _____ |
| 15. | ____ | Closes bellows knob | _____ |
| 16. | ____ | Deflates cuff 2 mmHg per second | _____ |
| 17. | ____ | Deflates cuff after 10 mmHg, after last audible sound heard | _____ |
| 18. | ____ | Records readings | _____ |
| 19. | ____ | Reads zero value | _____ |
| 20. | ____ | Observer holds arm vertical for full 15 seconds | _____ |
| 21. | ____ | Begins steps for next readings | _____ |

Certification Observer may discontinue monitoring at this point unless problems with a procedure is noted. If problems are noted, repeat monitoring beginning with Step 10.

Y-tube Stethoscope Observations: ____ /____ Initials ____ Date ___ / ___ / ___

____ /____ Initials ____ Date ___ / ___ / ___

Dates that the Random-Zero Sphygmomanometer was inspected:

## 5.8 Acknowledgment of Adaption

MDRD

Blood Pressure Measurement

Training and Quality Control

Adapted By

Robert P. Byington, Ph.D.

Adapted from the Procedures

of the Systolic Hypertension in the Elderly Program (SHEP)

January 1985

by Darwin R. Labarthe and Melanie Palmer

which were

Based on the Procedures
of the Hypertension Detection and Follow-up Program (HDFP)

by

Darwin R. Labarthe, M.D., Ph.D.

Sharon B. Poizner-Cooper, Ph.D.

Gary R. Cutter, Ph.D.

Barbara H. Casey, B.A.

Some text adapted from the ARIC Protocol 11:

"Sitting Blood Pressure and Postural Changes" (4/16/87)

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# MDRD
# BLOOD PRESSURE MEASUREMENT
# TRAINING AND QUALITY CONTROL

Date of Revision: September 1989

Adapted By:
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Department of Public Health Sciences
Bowman Gray School of Medicine
Winston-Salem, NC 27103

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## 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

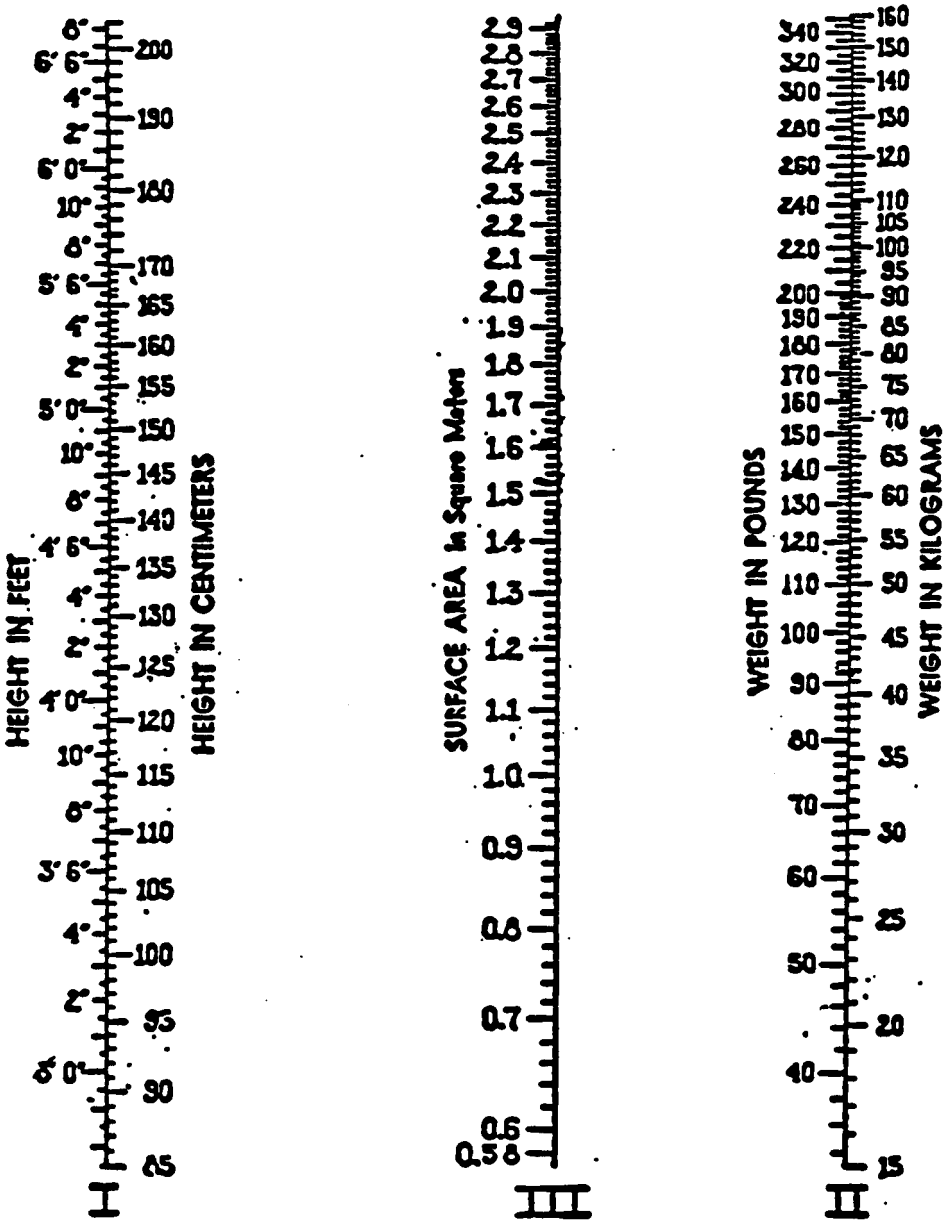
The serum activity for any given period will be the log mean of the serum sample counts at the beginning and end of that period. For example, the serum activity corresponding to URINE #1 is derived from BLOOD #0 and BLOOD #1.

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

All GFR measurements will be corrected to $1.73m^2$ using the most recent height measurement and the weight determined on the day of 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. 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 three). This will be performed using time-averaged serum counts. This GFR is the actual outcome value which is stored in the database as the patient GFR.

Figure 1
DUBOIS BODY SURFACE CHART
(As prepared by Boothby and Sandiford of the Mayo Clinic)




DIRECTIONS

To find body surface of a patient, locate the height in inches (or centimeters) on Scale I and the weight in pounds (or kilograms) on Scale II and place a straight edge (ruler) between these two points which will intersect Scale III at the patient's surface area.

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

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

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)

$P_1 = 10^{(Log(2299) + Log(2101))/2} = 2198$

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

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

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

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/$1.73m^2$

$P_3$: $\frac{10805 \times 2.18}{1727} \times \frac{1.73}{1.53} = 15.42$ ml/min/$1.73m^2$

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

Avg. GFR = 15.09

SD = 0.98

$CV = \frac{0.98}{15.09} \times 100\% = 6.4\%$

## 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 125I 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:

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

where W = weight in kilos

H = height in centimeters

In this example, BSA = 1.53 $m^2$ 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:

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

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

multiplyiny 1.73/BSA; GFR = (14.37)(1.73/1.53) = 16.25 ml/min/1.73m$^2$. Other period GFR valure 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 | 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

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

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

Calculations are indentical 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 MDRD Protocol defined GFR for entry and slope calculations.

# $^{125}$I-Sodium Iothalamate (Glofil)

The sole supplier of Glofil in the United States at the present time is Isotex Inc, Box 909, Friendswood, Texas 77546, phone (713) 482-1231. 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 1/2 ml plastic 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 <u>should not</u> be given the iothalamate.

MDRD 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. <u>Gloves should be worn in case dose leakage or other unsafe conditions arise.</u> All syringes, needles, empty isotope bottles and any other radioactive trash should be disposed of via established contaminated refuse protools (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.

## Dose Estimates

### 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 uCi 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/35uCi) | | |
|---|---|---|---|
| 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 $^{125}$I-iothalamate

| Organ | Estimated Radiation Dose | | | |
|---|---|---|---|---|
| | 2.0 hours ** | | 4.8 hours ** | |
| | mGy/MBq | rad/mCi | mGy/MBq | rad/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

Acknowledgement

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.

## Iowa Radiation Safety Committee Information
## Description of Project and Rationale

The Modification of Diet in Renal Disease (MDRD) Study has been designed to test the hypotheses that a reduction in dietary protein and phosphorus, and/or a reduction of blood pressure below the usual target of 140/90 mm Hg, may slow or stop the progression of chronic renal insufficiency. In addition, the MDRD Study will examine the nutritional safety and patient acceptance of low protein diets. To study these questions, patients with established renal disease and reduced renal function that has not yet progressed to the point where dialysis or a renal transplant is required, and who are otherwise in good health, will be asked to volunteer for a four year study. Consenting patients will undergo a three month period of dietary and blood pressure education and then will be randomized to one of three diets depending upon their initial glomerular filtration rate (GFR). Patients with an initial GFR between 25 and 60 ml/min will enter Study A and will be randomized to a diet with a relatively normal content of protein and phosphorus (Diet M) or to one containing approximately half this content of protein and phosphorus, but all as natural foods (Diet L). Patients with initial GFRs between 13 and 25 will be randomized either to Diet L (as above) or to Diet K, which contains a still more limited amount of protein and phosphorus, and which is given with a supplement of essential keto and amino acids. Patients will also be assigned randomly to one of two mean blood pressure control targets, 107 mm Hg (which is equivalent to the usual clinical blood pressure control goal of 140/90), or 92 mm Hg (which is equivalent to a blood pressure of 125/75, closer to the population average). Patients will then be followed monthly with careful attention to dietary compliance, to blood pressure control, and to nutritional status. Dietary compliance will be assessed by measurement of urinary urea excretion and by dietary records. Nutritional safety will be monitored by measurement of plasma proteins, body weight, and by anthropometric measurements. Dietary satisfaction will be assessed by compliance and by questionnaire.

The major outcome variable reflecting the impact of the various study treatments will be rate of change of GFR, as measured by urinary clearance of $^{125}$I-iothalamate. GFR will be measured at entry and at the end of the three month baseline period, two and four months later, and then every four months

until the end of the study. Since recruitment will be over a period of two years and the entire study will last four years, patients will have a minimum of 9 and a maximum of 15 GFR determinations (barring drop out or reaching a study stop point). Patients must be between the ages of 18 and 70 inclusive. Female patients will be excluded if they are pregnant or lactating or planning to become pregnant during the period of the study. Prior to each GFR determination, all women with the biological potential for pregnancy will have a determination of plasma HCG to exclude pregnancy. Urine flow rate is maximized by oral water loading, both to reduce radiation exposure and to maximize the precision of the GFR determination.

The MDRD Study is a multicenter clinical trial sponsored and approved by the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK) and supported by NIDDK and by the Health Care Financing Administration. It is anticipated that approximately 800 patients will be randomized in the entire study, and that about 53 patients will be randomized in each of the 15 clinical centers, of which the University of Iowa is one.

## Radiation Exposure to Subjects

A. Internal Source:

1. Compound administered: $^{125}$I-iothalamate (Glofil)
2. Source and Amount:
   a. Supplier: Isotex, Inc., Box 909 Friendswood, TX, (713) 482-1231
   b. Purity assurance: Checked by paper chromatography both by manufacturer and U of I Nuclear Medicine Dept.
   c. Amount administered/dose: 35 subcutaneous injection
   d. Number of doses/subject: 8 -14 depending upon time in study
   e. Pyrogen testing: No
3. Radiation Exposure/Dose (mrads):

| | Normal GFR | Near ESRD |
|---|---|---|
| a. Whole body: | 0.24 | 2.83 |
| b. Gonads (ovary) | 0.30 | 3.02 |
| c. Blood forming organs | 0.24 | 3.36 |
| d. Lens | N/A | N/A |
| e. Kidneys | 0.84 | 3.70 |
| f. Bladder wall | 29.82 | 32.41 |

g. Method of calculating the above data: Radiation dose is determined by renal function. Assumes voiding interval of 3.5 hr. Amount of kidney and bladder radioactivity calculated by assuming that nuclide is cleared by GFR alone. Whole body dose calculated by assuming distribution of nuclide in total body water. (For more details, see Iowa Radiation Dosimetry Section.)

4. Expected Urinary and/or fecal losses:
It is expected that essentially all of the administered nuclide will be excreted in the urine since it has no other known route of disposition or site of deposition in the body, especially after administration of SSKI to block thyroidal uptake of any free iodide.

5. Radiation Exposure/Total (mrads): (assuming a maximum 15 doses in 4 yrs.)

| | Normal GFR | Near ESRD |
|---|---|---|
| a. Whole body | 3.60 | 42.45 |
| b. Gonads (ovary) | 4.50 | 45.30 |
| c. Blood forming organs | 3.60 | 50.40 |
| d. Lens | N/A | N/A |
| e. Kidneys | 12.60 | 55.50 |
| f. Bladder wall | 447.30 | 486.15 |

**How do you propose to prevent an accidental overdose of radiation or radioactive material to the subject?**

Each dose will be drawn up in a syringe and prepared by a trained nuclear pharmacist in the Nuclear Medicine Pharmacy. The syringe will be counted prior to sending the dose for administration to the subject, as well as after administration of the dose.

**How will radioactive waste from the patient or subject be handled? What precautions will be taken to protect auxiliary personnel from radiation? How will they be trained?**

Aliquots of patient urine samples will be saved for counting; the remainder will be disposed of in the Clinical Research Center where the studies will be

conducted. Plasma and urine samples will be sent in appropriate containers to the central GFR laboratory at the Cleveland Clinic to be counted.

Auxiliary personnel will be protected by wearing radiation film badges to monitor their exposure. The GFR technician will wear gloves while handling the radionuclide dose.

The GFR nurses will be trained by training sessions conducted at the central GFR laboratory prior to study initiation, as well as by working with experienced research nurses in the Clinical Research Center. In addition, it should be mentioned that this radionuclide GFR technique has already been performed as a part of Phase II (Pilot Study) of the MDRD Study in the CRC. The present MDRD GFR nurse, who is now quite experienced in this technique, will perform half the clearance determinations and will assure the proper use of the nuclide by the second GFR nurse.

## Selection of Subjects

a. Criteria for subject selection:

Approximately 65 consenting individuals between the ages of 18 and 70 without insulin dependent diabetes, and with serum cretinine between 1.4 and 7.0 mg/dl (men) or 1.2 and 7.0 mg/dl (women), will be recruited from the clinics of the University or VA Hospitals or from cooperating clinicians in Iowa. We assume that 80% (52) of these 65 patients will be randomized into the follow-up phase. Specific exclusions are:

i. Pregnancy, lactation, or intention to have children before the end of the study.

ii. Nutritional disorders: weight < 80% or > 160% of standard body weight, serum albumin < 3.0 g/dl, or proteinuria > 10 g/day.

iii. Inability to give informed consent (prisoners, institutionalized mentally ill), or unwilling to consent.

iv. Known renal obstruction, known staghorn calculi, or inability to empty the bladder for whatever reason.

v. Serious medical diseases — uncontrollable blood pressure or edema.

vi. Drugs — known to affect renal function (NSAID) or INDs.

vii. Allergy to iothalamate or iodine

b. Age limitations:
Patients must be between 18 and 70 years old.

c. Limitations placed on female subjects to limit risk to potentially pregnant subjects.
Women with childbearing potential (premenopausal and not surgically sterilized) will only be studied after pregnancy has been excluded by performance of a sensitive blood test for human chorionic gonadotrophin. All women will have been told to tell the investigator if there is any chance that they are pregnant.

## Subject Consent

a. How will legal consent be obtained?
When a potential subject is identified, the entire study will be explained to him/her using the attached Subject Information Summary. Consent will be documented when the subject signs the Consent Form.

b. A copy of the Consent Form and the Patient Information Summary is attached.

c. How do you propose to meet [the requirement that absence of pregnancy be documented.]?
See Item 8c above.

d. Subjects below the age of consent.
Not applicable.

e. A copy of the Consent form and the Patient Information Summary is attached.

f. Will the patient be charged for this test or service?

## Pharmacological and Metabolic Considerations

a. Pharmacologic effects of the radionuclide.
125I-iothalamate is cleared essentially entirely by glomerular filtration. The nuclide will be used in tracer doses only, and no pharmacologic effects are anticipated.

b. Metabolic disposition.
The nuclide is quite stable and are excreted unchanged in the urine. It is distributed initially in the extracellular water, and is cleared from that space by GFR. It is not concentrated in any organ other than the kidney and urinary collecting system. It has a biological half time which is dependent upon the GFR, being approximately 100 min in normals, and up to 2000 min in patients with severe (but not end stage) renal insufficiency.

## Risk benefit ratio

a. Discuss the risk-benefit ratio.
Patients will benefit from close medical attention provided by the study. These benefits may include improved blood pressure control, better nutritional status, and a sense of pride from contributing to the better understanding of the best way to manage progressive renal disease. In addition, there is some economic incentive, in that medical care and the tests and medicines required by the study are provided to the participants at no cost.

The risks are considered to be small. They include the risks of adverse reactions to blood pressure medicines or of drug induced hypotension, and the possibilities of mild nutritional deficiencies. Either of these should be detected quickly by established monitoring methods. The risks also include a very small radiation exposure and a small dose of potassium iodide. The radiation to the bladder from the total of 15 GFR tests is no more than that delivered by 2 single abdominal x-ray films including the bladder (250 mrads each). Radiation to all other organs is no more than the dose absorbed from 2 PA chest x-rays (25 mrads each). In addition the

patients will have heparin locks placed, with the attendant minor risks of bruising and the remote possibility of infection.

b. Justify the use of radioisotopes.

Radioisotopes are now widely used with several techniques to measure GFR. These techniques have the advantage of being easy for both the patient and the physician to perform and analyze, and are relatively inexpensive. The radioisotopic determination of GFR is far more precise and accurate a measure of GFR than the creatinine clearance. The alternative method for determining GFR, use of inulin, is quite difficult presently because of a severe shortage of inulin for injection in the United States.

c. Discuss the importance of the study.

We believe that this is a very important study. Progressive renal insufficiency and the management of renal failure are major medical and socioeconomic problems. While it has been suggested that dietary therapy and very stringent blood pressure control may be beneficial, there have been no well constructed tests of these hypotheses. Dietary therapy both is expensive and imposes a considerable burden on patients. Both dietary therapy and reduction of blood pressure to low targets may have unrecognized risks. This study should resolve these major questions.

**If this proposal involves the administration of (or exposure to) more than one radiation dose, a flow chart outlining each exposure should be described below.**

Each subject will receive only one radiation dose during each study. However, each subject will be studied between 9 and 15 times: on entry and at the end of the baseline period and 2, 4, 8, and 12 months later, and then every four months for the duration of the study (two to four years for any given patient), and finally at the end of the study.

## IOWA RADIATION DOSIMETRY SECTION

The calculations for the radiation dosimetry of 125I-iothalamate are simplified by the strong evidence that it is distributed in the extracellular

water and is found only in the kidneys and the urinary tract in concentrations above that in blood (1), from which it is cleared by the kidneys in accordance with a double exponential curve. Thus the problem in dosimetry consists in finding the organ dose attributable to the presence of the radiopharmaceutical in blood and the fraction from the urinary tract. The former is a relatively simple problem, but the latter is complicated by the variability of the dose to the pelvic organs from kidney and bladder activity, depending upon renal transit time and the frequency with which the bladder is emptied. Smith et al (2) have studied this question in detail and have provided estimates for the organ doses of a number of renal radiopharmaceuticals including 125I-iothalamate, making the assumptions of normal renal function, body weight of 70 kg, and an estimated interval between bladder emptyings of 3.5 hours. Further, estimates of the fraction of the total dose contributed by the kidney and bladder (K+B) were given. The contribution from the whole body (WB) distribution can be determined by subtraction. Table I gives these estimates for a patient with normal renal function who receives 35 uCi of $^{125}$I-iothalamate, converted to the more familiar units, mrads.

**Table I. Dosimetry of 35 uCi of $^{125}$I-iothalamate Assuming Normal Renal Function.**

Assumes a GFR Of 97 ml/min, body weight of 70 kg, and voiding interval of 3.5 hrs. K+B = dose derived from kidney and bladder nuclide. WB = "whole body" dose from distribution of nuclide in whole body water. Tot = total organ dose. Dose given in mrads.

| | Embryo | Ovaries | Marrow | Kidneys | Bladder wall | Total body |
|---|---|---|---|---|---|---|
| K+B | 0.72 | 0.15 | 0.07 | 0.69 | 29.7 | 0.10 |
| WB | 0.15 | 0.15 | 0.17 | 0.15 | 0.1 | 0.14 |
| Total | 0.87 | 0.30 | 0.24 | 0.84 | 29.8 | 0.24 |

The above estimates are not valid when renal clearance (GFR) is reduced. Corrected estimates can, however, be calculated from the above figures. The "whole body" dose is directly proportional to the effective half time of the radiopharmaceutical, which is a function of the biological half time (in this case determined by GFR) and the physical half life of the radionuclide.

$$T1/2\ eff = \frac{(T1/2\ biol)(T1/2\ phys)}{(T1/2\ biol) + (T1/2\ phys)}$$

Assuming that the normal T1/2 biol of a marker of GFR is 100 min (equivalent to a GFR of 97 mm/min in a 70 kg person) in a person with normal GFR, and is 2000 min (equivalent to a GFR of 4.85 ml/min in a person with severe renal insufficiency but not yet on dialysis, one can calculate that the "whole body" dose for $^{125}$I-iothalamate will be increased 19.5 fold in the patient with severe renal insufficiency.

On the other hand, the radiation dose contributed by the radionuclide concentrated within the kidney and bladder is a function of the fraction of the administered nuclide that is excreted by that route. For 125I-iothalamate, virtually the entire dose (98%) will be excreted through the kidneys even in the presence of severe renal inusfficiency, since nuclide decay in the relevant period is insignificant. Thus the estimates of the K+B dose do not have to be modified.

Table II contains radiation dose calculations based upon the above two paragraphs for a patient with a GFR of 5 ml/min, or 5% of normal. The dose for patients with intermediate degrees of renal insufficiency will fall between the two extremes presented in Tables I and II.

Table II. Dosimetry of 35 uCi of $^{125}$ I-iothalamate Assuming GFR of 5 ml/min.

Remainder of assumptions as in Table I.

| | Embryo | Ovaries | Marrow | Kidneys | Bladder wall | Total body |
|---|---|---|---|---|---|---|
| K+B | 0.72 | 0.15 | 0.07 | 0.69 | 29.7 | 0.10 |
| WB | 2.87 | 2.87 | 3.29 | 3.01 | 2.7 | 2.73 |
| Total | 3.59 | 3.02 | 3.36 | 3.70 | 32.4 | 2.83 |

Finally, it should be noted that the dose contributed by the isotope in the kidneys and bladder to each organ other than the bladder will be reduced roughly in proportion to the dwell time in the bladder. As it is anticipated that

patients in this study will be voiding on average 2 - 3 times per hour, the estimates of irradiation from the kidney and bladder isotope are overestimates. Further the anticipated high volume of urine flow should substantially reduce the dose to the bladder by decreasing the concentration of the nuclide in the bladder. The calculations in reference 2 were made assumeing a urine flow rate of 1 ml/min, whereas we shall try to achieve flow rates in excess of 3 ml/min. The true bladder dose may therefore be reduced by a factor of almost three.

## REFERENCES

### Iowa Radiation Dosimetry Section

1. McAfee JG, Gagne G, Atkins HL, et al. Biological distribution and excretion of DTPA labeled with Tc-99m and In-111. J Nucl Med 20: 1273-1278, 1979.

2. Smith T, Veall N, and Altman, DG. Dosimetry of renal radiopharmaceuticals: the importance of bladder radioactivity and a simple aid for its estimation. Brit J Radiol 54: 961-965, 1981.

APPENDIX III

## Central GFR
## "Supply Order Form"

REQUEST FOR MDRD SUPPLIES:

Center Number _____ Date of Request _____

Please check supplies needed and return this form to the GFR Central Lab in your next sample mailing. We will send the supplies when we return your mailer. Please notify us before you run out completely. Thanks.

| | | |
|---|---|---|
| ☐ Packing Tape | ☐ GFR Tube Labels | ☐ "Radioactive" Tape |
| ☐ Polar Packs | ☐ Ziplock Bags | ☐ Other (Specify below) |
| | ☐ Sample Tubes: | |

Specify Size _____ Specify Color _____

Comments: _____

_____

_____

_____

_____

Date Supplies Mailed _____

GFR Lab Person _____

## 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 MDRD GFR procedure:

1. An error in isotope administration may be identified by clinical center personnel or by staff of the Central GFR Laboratory. (Errors should be reported to the Quality Control Committee.) Errors which result in misadministration of more than the prescribed dose must be reported immediately to the Clinical Management 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 Clinical Management 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 Clinical Management Committee must be notified, in writing, of the determination. If a misadministration 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 Clinical Management 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 Clinical Management 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 Patient Safety Committee within three months of the occurrence or notification of the misadminstration (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 patient's 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 Clinical Management 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.

# APPENDIX V

## Alternative Marker for GFR

# Biochemistry's List of Test Methods/Instrumentation Codes

## CALCIUM - SERUM (MG/DL)

A. ALIZARIN
- 01 Baker Centrifichem
- 02 Baker Encore
- 03 All Auto Chem Instr

B. ARSENAZO III DYE
- 01 Kodak Ektachem
- 02 All Auto Chem Instr

C. ATOMIC ABSORPTION
- 01 All Auto Chem Instr
- 02 All Atomic Absorp Spec

D. CHLOROPHOSPHONAZO III
- 01 All Auto Chem Instr

E. CRESOLPHTHALEIN COMPLEXONE
- 01 Abbott ABA 100
- 02 Abbott ABA 200
- 03 Abbott Spectrum
- 04 Abbott VP
- 05 American Dade Paramax
- 06 American Mon. Parallel
- 07 American Mon. KDA
- 08 Baker Centrifichem
- 09 Beckman Astra 4 & 8
- 10 BM Diag. 8700/M
- 11 Chemetrics II
- 12 Coulter Dacos
- 13 Dow
- 14 Dupont ACA
- 15 Electronuc Flexigem
- 16 Electronuc Gemeni
- 17 Electronuc Gemstar
- 18 Gilford Impact 400, Etc.
- 19 Gilford Sys 102, Etc.
- 20 Gilford Sys 103, 202, 5
- 21 Hitachi 705 (BMD)
- 22 Hitachi 737 (BMD)
- 23 IL Multistat III
- 24 Il 508/504
- 25 Kone Instruments
- 26 Olumpus Demand
- 27 Roche Cobas
- 28 Roche Cobas Mira
- 29 Technicon RA 1000
- 30 Technicon SMA 12/60
- 31 Technicon SMAC
- 32 All Auto Chem Instr
- 33 All Manual Chem Instr

F. METHYLTHYMOL BLUE
- 01 IL Multistat III
- 02 All Auto Chem Instr

G. 00 OTHER METHOD, SPECIFY

_____

H. 00 TEST NOT PERFORMED IN THIS LAB

## CREATININE - SERUM (MG/DL)

A. ALK PICRATE W/ LLOYDS
- 01 Electronuc Gemeni
- 02 All Auto Chem Instr
- 03 All Chem Instr

B. ALK PICRATE W/O LLOYDS
- 01 Electronuc Gemeni
- 02 Electronuc Gemstar
- 03 Gilford Impact 400, Etc.
- 04 Gilford Sys 102, Etc.
- 05 Gilford Sys 103, 202, 5
- 06 Manual, In House Reag.
- 07 Olympus Demand
- 08 Technicon RA 1000
- 09 Technicon SMA 12/60
- 10 Technicon SMAC
- 11 All Auto Chem Instr
- 12 All Manual Chem Instr

C. ENZYMATIC
- 01 Kodak Ektachem
- 02 All Auto Chem Instr

D. KINECTIC ALK. PICRATE
- 01 Abbott ABA 100
- 02 Abbott ABA 200
- 03 Abbott Spectrum
- 04 Abbott VP
- 05 American Dade Paramax
- 06 American Mon. Parallel
- 07 American Monitor KDA
- 08 Baker Centrifichem
- 09 Beckman Astra 4 & 8
- 10 Beckman Sp Const Analy
- 11 BM Diag. 8700/M
- 12 Chemetrics II
- 13 Coulter Dacos
- 14 Dupont ACA
- 15 Electronuc Gemeni
- 16 Electronuc Gemstar
- 17 Gilford Impact 400, Etc.
- 18 Gilford Sys 102, Etc.
- 19 Gilford Sys 103, 202, 5
- 20 Hitachi 705 (BMD)
- 21 Hitachi 737 (BMD)
- 22 IL Multistat III
- 23 IL 508/504
- 24 Kone Instruments
- 25 Olympus Demand
- 26 Roche Cobas
- 27 Roche Cobas MIRA
- 28 Technicon RA 1000
- 29 All Auto Chem Instr

E. 3, 5 DINITRO BENZOIC
- 01 Ames Seralyzer

F. 00 OTHER METHOD, SPECIFY

_____

G. 00 TEST NOT PERFORMED IN THIS LAB

## Biochemistry's List of Test Methods/Instrumentation Codes

MAGNESIUM - SERUM (MG/DL)

A. ATOMIC ABSORPTION
- 01 IL AA Spectro
- 02 Perkin-Elmer
- 03 All Auto Chem Instr

B. CALMAGITE
- 01 Abbott VP
- 02 American Dade Paramax
- 03 American Mon. Parallel
- 04 American Monitor
- 05 American Monitor KDA
- 06 Baker Centrifichem
- 07 Electronuc Flexigem
- 08 Electronuc Gemeni
- 09 Gilford Impact 400, Etc.
- 10 Gilford Sys 102, Etc.
- 11 Hitachi 705 (BMD)
- 12 Olympus Demand
- 13 Pierce
- 14 Roche Cobas
- 15 Technicon RA 1000
- 16 All Auto Chem Instr
- 17 All Manual Chem Instr

C. COLORIMETRIC - METHYLTHY
- 01 Dupont ACA
- 02 All Auto Chem Instr

D. MAGNON
- 01 Gilford Impact 400, Etc.
- 02 All Auto Chem Instr

E. 00 OTHER METHOD, SPECIFY _____

F. 00 TEST NOT PERFORMED IN THIS LAB

UREA - SERUM (MG/DL)

A. CONDUCTIVITY RATE
- 01 Beckman Astra 4 & 8
- 02 Beckman Sp Const Analy
- 03 All Auto Chem Instr

B. DIACETYL MONOXIME
- 01 Dow
- 02 Technicon SMA 12/60
- 03 Technicon SMAC
- 04 All Auto Chem Instr
- 05 All Manual Chem Instr

C. O-PHTHALALDEHYDE
- 01 American Mon. Parallel
- 02 American Monitor KDA
- 03 Ames Seralyzer
- 04 All Auto Chem Instr
- 05 All Manual Chem Instr

D. UREASE HYDROLYSIS
- 01 Beckman Astra 4 & 8
- 02 Electronuc Gemeni
- 03 Olympus Demand
- 04 All Auto Chem Instr

UREA - SERUM (CONT)

E. UREASE INDOPHENOL
- 01 All Auto Chem Instr
- 02 All Manual Chem Instr

F. UREASE QUINOLINIUM
- 01 Kodak DT 60
- 02 Kodak Ektachem
- 03 All Auto Chem Instr

G. UREASE WITH GLDH
- 01 Abbott ABA 100
- 02 Abbott ABA 200
- 03 Abbott Spectrum
- 04 Abbott VP
- 05 American Dade Paramax
- 06 Baker Centrifichem
- 07 Beckman Astra 4 & 8
- 08 BM Diag. 8700/M
- 09 Chemetrics
- 10 Chemetrics II
- 11 Coulter Dacos
- 12 Dupont ACA
- 13 Electronuc Flexigem
- 14 Electronuc Gemeni
- 15 Electronuc Gemstar
- 16 Gilford Impact 400, Etc.
- 17 Gilford Sys 102, Etc.
- 18 Gilford Sys 103, 202, 5
- 19 Hitachi 705 (BMD)
- 20 Hitachi 737 (BMD)
- 21 IL Multistat III
- 22 IL 508/04
- 23 Kone Instruments
- 24 Olympus Demand
- 25 Roche Cobas
- 26 Roche Cobas MIRA
- 27 Technicon RA 1000
- 28 All Auto Chem Instr
- 29 All Manual Chem Instr

H. 00 OTHER METHOD, SPECIFY _____

I. 00 TEST NOT PERFORMED IN THIS LAB

_____
Signature of Lab Director

_____
Date

_____
Institution

## Table 3.3.1 Sample Requirements and Number of the Tubes to be Drawn at Each Visit

| Visits | BV 0 | BV 1 | BV 2 | BV 3 | 1 25 | 2[a] 26 | 3 27 | 4 28 | 5 29 | 6d 30 | 7 31 | 8 32 | 9 33 | 10 34 | 11 35 | 12 36 | 13 37 | 14 38 | 15 39 | 16 40 | 17 41 | 18 42 | 19 43 | 20 44 | 21 45 | 22 46 | 23 47 | 24 48 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| # of 7cc Vacutainer tubes (red Top) | | | | | | 1 | | | | 1 | | | | 1 | | | | 1 | | | | 1 | | | | 1 | | |
| # of 10cc Vacutainer tubes (red top) | 1 | | | 3 | | 1 | | 3 | | | | 3 | | | | 3 | | | | 3 | | | | 3 | | | | 3 |
| Total # of mls of serum needed by CBL | 5 | | | 13 | | 5 | | 13 | | 3 | | 13 | | 3 | | 13 | | 3 | | 13 | | 3 | | 13 | | 3 | | 13 |
| # of 5cc EDTA Vacutainers (purple top) | | | | 1 | | | | 1 | | | | 1 | | | | 1 | | | | 1 | | | | 1 | | | | 1 |
| # of Heparin Vacutainers (green top) for Amino Acids | | | | 1 | | 1b | | 1b | | | | 1 | | | | 1b | | | | 1 | | | | 1b | | | | 1 |
| 24 hour Urine Aliquot | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| GFR c with Visit | √ | | | √ | | √ | | √ | | | | √ | | | | √ | | | | √ | | | | √ | | | | √ |
| Local Laboratory Require-ments | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

a. 2 month visit requires one 10cc tube (5 ml serum) and a GFR; 26 month visit requires one 7cc tube (3 ml serum) and no GFR.

b. Draw Heparin tubes with this visit ONLY IF patient is on Diet K.

c. Patient should be fasting for any visit requiring a GFR or Lipid testing.

d. Study F and G visits require same amount of serum as Follow-Up 6 Visit.

## Frequency and Location of Laboratory Testing

**Study F**

| | Month # | | | | | |
|---|---|---|---|---|---|---|
| | 4 | 8 | 12 | 16 | 20 | 24* |
| Creatinine | C | C | C | C | C | C |
| Albumin | C | C | C | C | C | C |

*Study F visits continue at 4 month intervals.

C=Central Biochemistry Laboratory

## APPENDIX IX

### Reference Ranges for the Central Biochemistry Laboratory

| Serum | Sex | Range | Units |
|---|---|---|---|
| Transferrin | | 210 to 375 | mg/dl |
| Albumin | | 3.7 to 4.9 | g/dl |
| Phosphorus | | 2.5 to 4.5 | mg/dl |
| Creatinine | | 0.7 to 1.4 | mg/dl |
| Urea Nitrogen | M | 10 to 25 | mg/dl |
| Urea Nitrogen | F | 8 to 25 | mg/dl |
| Uric Acid | F | 2.0 to 7.0 | mg/dl |
| Uric Acid | M | 3.0 to 8.0 | mg/dl |
| T. Bilirubin | | 0.0 to 1.5 | mg/dl |
| AST (SGOT) | | 7 to 40 | IU/L |
| LDH | | 50 to 210 | IU/L |
| Triglycerides | | 30 to 200 | mg/dl |

| Whole Blood | Range | Units |
|---|---|---|
| Hemoglobin A1c | 3.5 to 6.5 | % |

| 24 hr. Urine | Sex | Range | Units |
|---|---|---|---|
| Creatinine | M | 1000 to 2000 | mg/day |
| Creatinine | F | 800 to 1800 | mg/day |
| Urea Nitrogen | | 12 to 20 | g/day |
| Protein | | < 0.15 | g/day |
| Phosphorus | | 900 to 1300 | mg/day |
| Sodium | | 40 to 220 | mEq/day |
| Potassium | | 30 to 99 | mEq/day |

## APPENDIX X

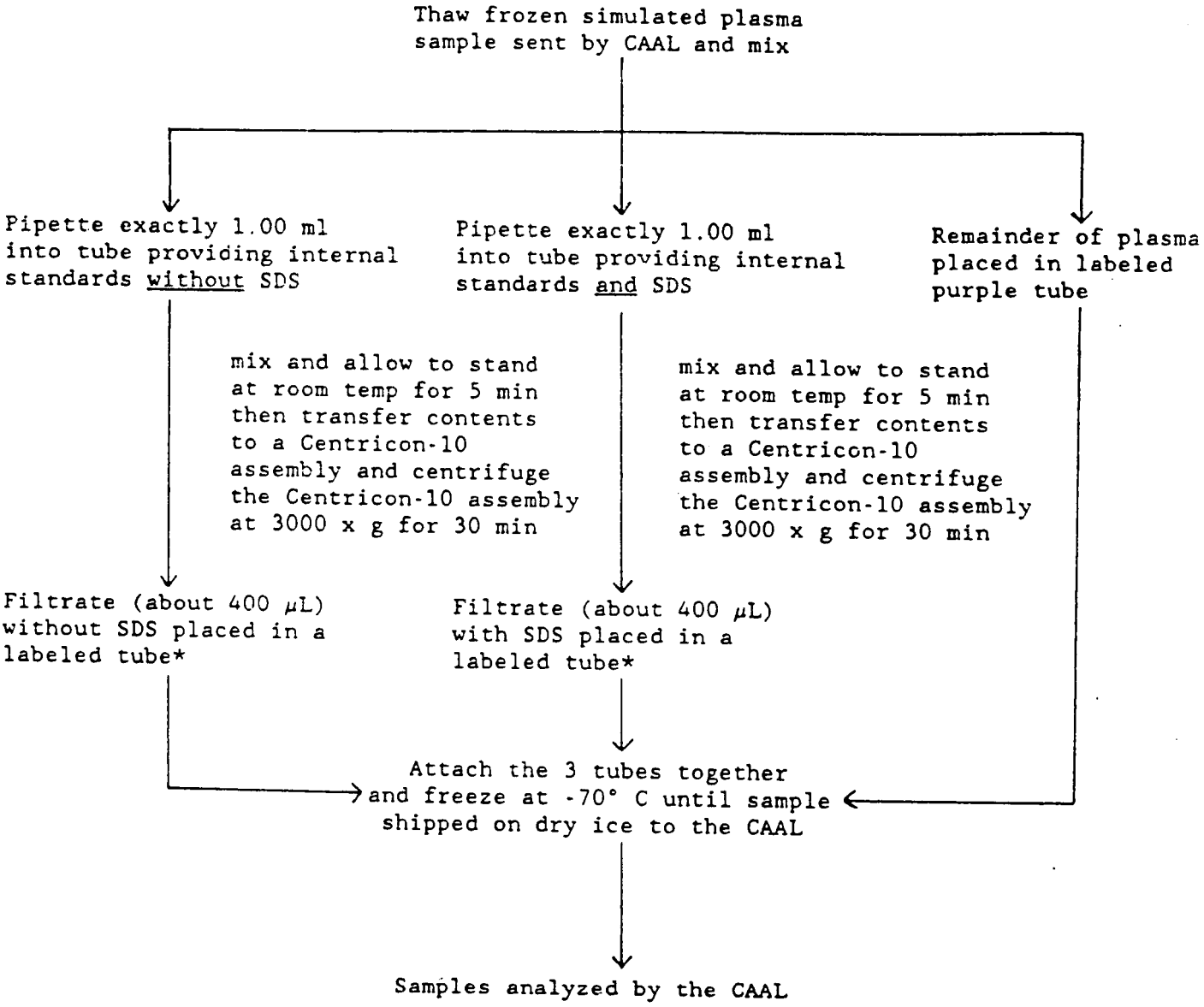
## LIPID REFERENCE RANGES

| | Age | Sex | Risk Classification: Desirable | Borderline /High | High |
|---|---|---|---|---|---|
| Total Cholesterol | <20 | M,F | 75 - 169 | 170 - 199 | ≥200 |
| | ≥20 | M,F | 100 - 199 | 200 - 239 | ≥240 |
| LDL-Cholesterol | <20 | M,F | 50 - 99 | 100 - 129 | ≥130 |
| | ≥20 | M,F | 60 - 129 | 130 - 159 | ≥160 |
| HDL-Cholesterol | All | M | >45 | 36 - 45 | ≤35 |
| | All | F | >55 | 36 - 55 | ≤35 |
| HDL-2-Cholesterol | All | M | >14 | 10 - 14 | ≤10 |
| | All | F | >20 | 10 - 20 | ≤10 |
| HDL-3-Cholesterol | All | M | >31 | 25 - 31 | ≤25 |
| | All | F | >35 | 25 - 35 | ≤25 |
| VLDL-Cholesterol | <15 | M,F | 4 - 20 | 21 - 49 | ≥50 |
| | ≥15 | M,F | 5 - 33 | 34 - 82 | ≥83 |
| Triglycerides | <15 | M,F | 25 - 120 | 121 - 299 | ≥300 |
| | ≥15 | M,F | 30 - 200 | 201 - 499 | ≥500 |
| Apolipoprotein A-I | ≥20 | M | >127 | 110 - 127 | <110 |
| | ≥20 | F | >147 | 127 - 147 | <127 |
| Apolipoprotein B | ≥20 | M | <98 | 98 - 112 | >112 |
| | ≥20 | F | <92 | 92 - 112 | >112 |
| A-I/B Ratio | ≥20 | M | >1.30 | 0.98 - 1.30 | <0.98 |
| | ≥20 | F | >1.60 | 1.13 - 1.60 | <1.13 |

### Risk of Developing Coronary Heart Disease and the Total Cholesterol/HDL Cholesterol

| | Risk | TC/HDL-C Ratio |
|---|---|---|
| Men | 1/2 average | 3.43 |
| | average | 4.97 |
| | 2x average | 9.55 |
| | 3x average | 23.99 |
| Women | 1/2 average | 3.27 |
| | average | 4.44 |
| | 2x average | 7.05 |
| | 3x average | 11.04 |

Figure 2: Scheme for handling quality control sample at the Clinical Centers

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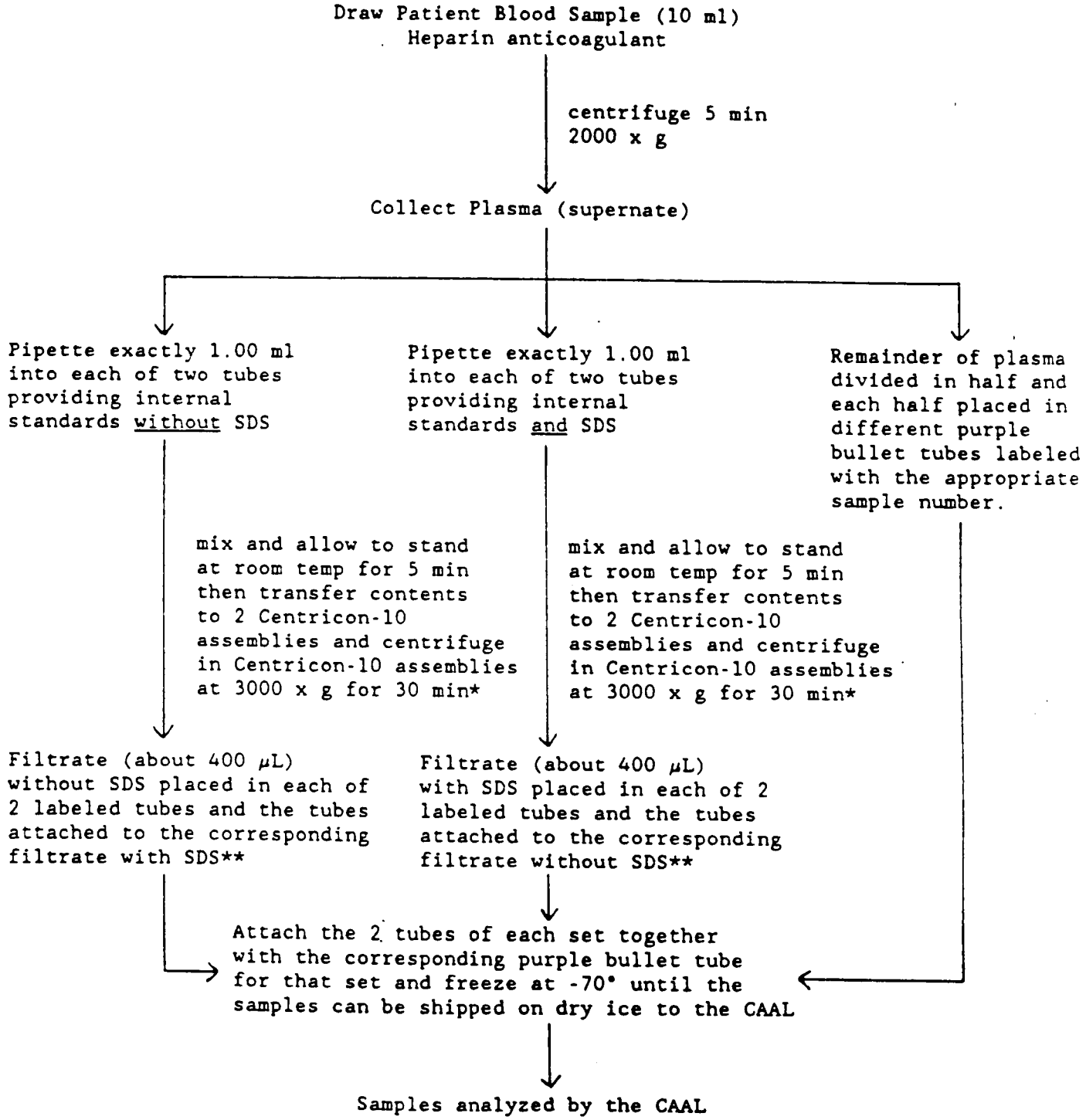
Abbreviations: SDS, sodium dodecylsulfate; CAAL, Central Amino Acid Laboratory.

*Note: Centricon-10 ultrafilters are discarded after use.

Figure 3: Scheme for sample handling of external quality control specimens at the Clinical Centers

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Abbreviations: SDS, sodium dodecylsulfate; CAAL, Central Amino Acid Laboratory.
*Note: Centricon-10 ultrafilters are discarded after use.
**Filtrate cups may be capped, labeled and sent directly.

## Calculating "g force" for Amino Acid Centrifuge

Five ml blood for analysis of plasma amino acids and alloisoleucine will be collected into tubes containing dry heparin. The blood will be quickly centrifuged in a clinical centrifuge at 2200 times gravity (2200 xg) for 15 minutes at 4C using a swing bucket rotor. The number of rotations per minute (rpm) necessary to generate this centrifical force will vary depending on the size of the rotor used and can be calculated as follows:

$$rpm = \frac{2200}{0.00001118 \times r}$$

where rpm = speed of rotation (rotations per minute)

r = distance from the center of the rotor around which the tube is centrifuged to the bottom of the centrifuged tube in the horizontal position (measured in centimenters).

Each center should perform this calculation and send the numbers used to the Central Amino Acid Laboratory for checking.

## REFERENCES


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## APPENDIX XIV

### Central Biochemistry Laboratory
### "Supply Order Form"

Center Number _____ Date of Request_____

Please check supplies needed and return this form to the CBL in your next sample mailing. We will send the supplies when we return the sample mailer. Please notify us before you run out completely.

- [ ] Packing Tape
- [ ] Ziplock Bags
- [ ] Polar Packs
- [ ] 30 ml Sample Tubes (Urine)
- [ ] 15 ml Sample Tubes (Serum)
- [ ] 7 ml Sample Tubes (Whole Blood)
- [ ] Other (Specify):_____

Comments:_____

_____

_____

Date Supplies Mailed:_____

## APPENDIX XV

Abbott TestPack HCG-COMBO Pregnancy Test Instructions

1. Remove testpack from wrapper.
2. Fill sample pipet to first indentation, with serum.
3. Pipet serum onto 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 MDRD Steering Committee for serum only. Urine samples are unacceptable.

### 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.

### STORAGE OF KIT

Reagent A and serum controls must be stored refrigerated at 2-8 degrees Celsius. 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.

APPENDIX XVI

## Sample Requirements for Action Items Analyzed by the CBL

| | Amount of Serum |
|---|---|
| Transferrin | 1.0 mL |
| Albumin | 1.0 mL |
| Phosphorus | 1.0 mL |
| * Cholesterol | 1.0 mL |
| * Triglyceride | 1.0 mL |
| * LDL Cholesterol | 3.0 mL |

* If any combination of these three tests is requested, a total of 3.0 mL of serum will be sufficient.

If an action item is ordered at a visit when other CBL serum tests are already required, the written amount of additional serum needed will be less than written on the table above. In this case, the technician will need to call the CBL for sample requirements.

## APPENDIX XVII

### Sample Requirements for Stop Points and Follow-up after Stop Point Analyzed by the CBL

Stop Point - 8ml of serum
1 ml of whole blood (EDTA)

* Follow-up After Stop Point - 3 ml of serum, and aliquot of 24-hour urine sample

* Not applicable for patients on dialysis or after tranplantation

## APPENDIX XVIII

## 24-HOUR URINE CHECKLIST

Patient 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 questions 1-9, then give the checklist to the dietitian before the dietitian sees the patient. The dietitian adds any comments based on discussion with the patient and returns the checklist to you to file in the patient's chart. The criteria for completing question 9 are found in the Manual of Operations, Volume I, Chapter 3, page 1.3.22. Note: This checklist is not a form. It is not entered into Datalex.

**Patient Followed:**

| Correct Procedures | Incorrect Procedures | |
|---|---|---|
| ____ | ____ | 1. Did the jug contain any liquid (even a small amount) before you started the 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 23.5 hours and no more than 24.5 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 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 emptied his 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, ate 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 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_____

10. Dietitian Comments:_____

_____