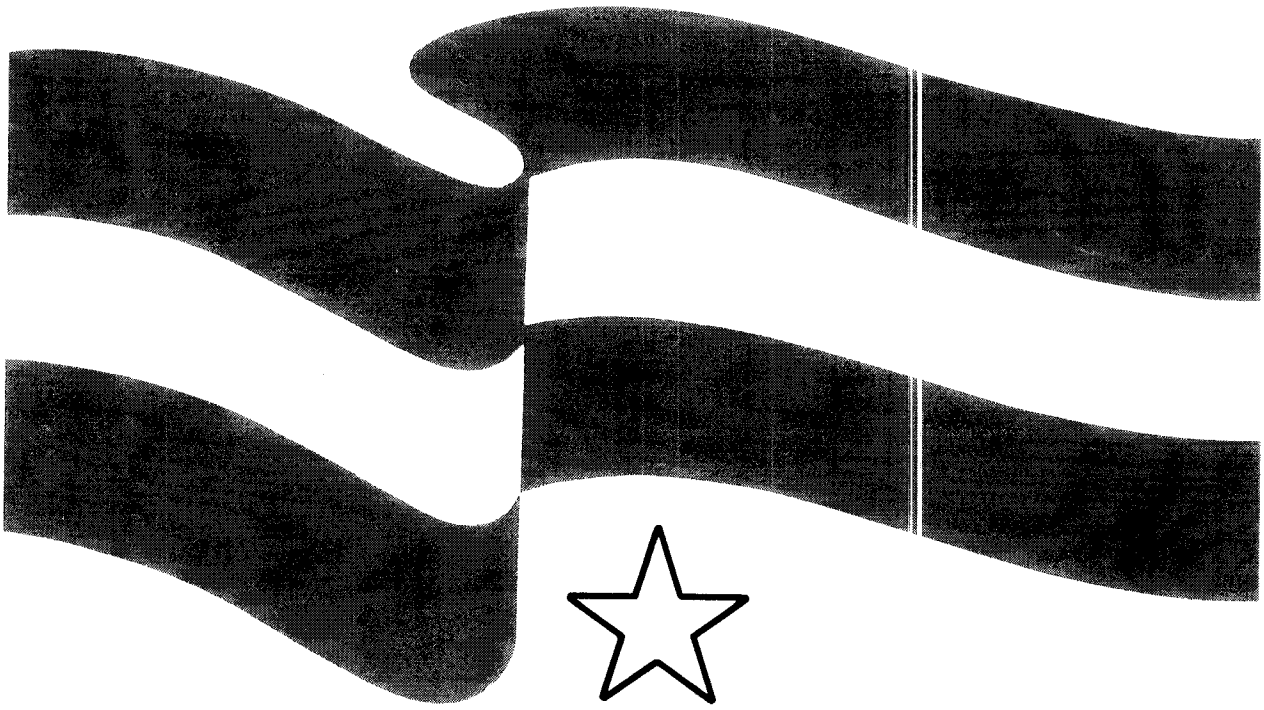

HEALTH STATUS OF VIETNAM VETERANS

SUPPLEMENT A LABORATORY METHODS AND QUALITY CONTROL

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
Centers for Disease Control

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LABORATORY METHODS AND QUALITY CONTROL

The Centers for Disease Control
Vietnam Experience Study
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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

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SUMMARY

This supplement provides information on the standardized procedures and methods used to perform a battery of laboratory assessments on blood, urine, and semen specimens obtained from veterans who participated in the medical examination component of the Vietnam Experience Study (VES). It includes detailed protocols for data management, specimen collection and processing, analytic methods and quality control (QC) procedures. In the final section, QC data obtained over the 16-month examination period are summarized.

The QC program used in this study was considerably more demanding than what is considered normal for a clinical laboratory. Each analytic run (usually including 23 participant samples per assay per day) contained "bench" and "blind" repeat QC samples. Bench controls were used to monitor precision of measurement within and among days over the course of the study. Blind repeat controls (*i.e.*, the technician was unaware of the cohort status of the donor) were used to monitor the repeatability of the measurement of a participant sample within the same analytic run. QC data from bench controls are summarized in this supplement. QC data from blind repeat controls are summarized in Supplement B, *Medical and Psychological Data Quality.*

We implemented a statistical QC program to monitor the quality of laboratory data obtained over the study period. Each respective technician maintained QC charts at the bench. In addition, two full-time QC supervisors and a board-certified clinical pathologist reviewed the participant and QC data daily. To aid them in this labor-intensive process, a customized statistical QC program was put into use on a personal computer to identify potential "out-of-control" runs. Criteria for acceptable performance of daily runs were based on a contractual agreement with the Centers for Disease Control. In that contract, performance guidelines were given for each bioassay. These guidelines were closely followed. Ultimately, however, the laboratory director decided whether the performance was acceptable.

The QC data were sent to CDC weekly by telephone. Data were then carefully checked for completeness, validity, and accuracy. We ran computer programs on these data as they accumulated. To check for long-term trends, we provided monthly computer-generated QC monitoring reports to Lovelace Clinical Laboratory for review there.

In conclusion, our overall review of QC data for these laboratory determinations suggests, that the bioassays, with one exception (the melioidosis titre), were performed with good precision and have provided high-quality laboratory data for use in assessing the current health status of veterans participating in the VES.

TABLE OF CONTENTS

I. INTRODUCTION

This manual provides documentation of the laboratory methods used and the QC results obtained for the bioanalysis of blood, urine, and semen specimens from veterans who participated in the Vietnam Experience Study. These analyses were performed by personnel in the Clinical and Research Division, Department of Laboratories, Lovelace Medical Foundation, Albuquerque, New Mexico.

All laboratory procedures were accomplished by using standardized protocols. In Section II, we describe the data management practices used in the laboratory to ensure the accuracy and integrity of the data. Specimen collection, handling, and processing are described in Sections III and IV. Analytical methods for each assay are described in Section V, and QC procedures and criteria are presented in Section VI. Finally, in Section VII, we summarize the QC data over the entire period of laboratory analyses.

Blood, random urine, and 12-hour urine specimens were obtained from 1462 study participants examined from June 1985 through September 1986. Specimens were collected on the morning of the physical examination and carefully transported to a processing area. Specimens were then delivered to various laboratory benches for analysis, usually within the next 24 hours. Calibration of equipment was verified daily. A statistical QC program was used to monitor within- and among-day variation for each assay. Technologists were responsible for maintaining QC charts and worksheets and problem logs at the bench. Participant and QC data were reviewed daily by two QC supervisors and a laboratory director. Weekly, QC data were transmitted electronically to Agent Orange Projects in Atlanta, Georgia, for further statistical evaluation of trends over time. These data are presented in Section VII.

Semen specimens were obtained from 571 study participants examined from May 1986 through September 1986. Specimens were processed within 2 hours after they were collected. Videotapes and microslides of sperm preparations were made for analysis of sperm concentration, motility, morphology, and morphometry by using a computer system for image analysis. Calibration of equipment was checked daily, and statistical QC was employed similarly to that for other bioassays. QC data are presented in Section VII.

Technologists were thoroughly trained by two full-time QC supervisors in the areas of computer data entry, analytical methods, and laboratory QC before performing analyses for this project. Only certified medical technologists and medical laboratory technicians were employed. Both QC supervisors had at least 10 years of experience in clinical laboratory work and management. The laboratory director was a Board-certified clinical pathologist. A Ph.D. statistician and a clinical chemist from CDC periodically visited the site to review laboratory techniques and data and to ensure consistency in the quality of the laboratory results.

In this manual, the use of trade names is for identification only and does not constitute endorsement by the Public Health Service or the U.S. Department of Health and Human Services.

II. LABORATORY DATA MANAGEMENT MANUAL

A. General Overview

1. Goal

The goal of the laboratory is to collect, process, and report accurate, complete participant and quality control (QC) data. An IBM-PC-XT, with the Community Health Computer (CHC) data management system on the laboratory mainframe computer system, provides the facility to capture, analyze, review, and transmit both of these types of data.

2. System Description

The laboratory hardware configuration consists of a Mod Comp Classic Model 7830 CPU, which controls the interpretation and execution of all instructions, monitors all on-line instruments, and handles all communications to and from remote stations. In addition, there is disc storage of 134MB, two tape drives, two line printers, and an operating console. The capacity and structure of the hardware is designed for application to both communications and scientific information processing.

The Mod Comp Classic uses the Max IV operating system and supports the advanced hardware features of the Classic CPU, including all context register files and peripheral devices. All application programs are written in ANSI Fortran IV; central facility programs execute in the background and remote station programs execute in the foreground.

Incorporated into the laboratory information system (LIS) is software to download QC data to an IBM-PC-XT for evaluation by using a customized statistical QC program written by Jerry Gentry of CDC. With the telecommunications software Crosstalk, QC data can be directly transmitted weekly, by telephone line, to CDC for analysis and review.

3. Data Collection Procedure

Laboratory personnel use a general purpose manual entry function from a microcomputer terminal (CRT) as the basic entry mode. Results are entered into the LIS in an unverified state, and data are manually checked for validity. In some cases, interfaced instruments transmit data in a unverified state directly from the instrument to the mainframe. A manual check is then performed to validate the results by using a double review technique.

B. Data Flow

1. Test Ordering Entry System

a. Participants' test requests

(1) Laboratory assistants in specimen processing enter the orders.

(2) About 1 week before the participants arrive, the Clinical Data Management Section provides the laboratory a list of participants.

(3) With the LIS function "AE," the participant is admitted to the system, tests are requested, and the collection date is specified.

(4) Participant data to be used for the study are --

(a) Participant number - seven digits, unique for each participant
(b) Participant name defined as AOP XXX, mmddyy
where XXX = subject's initials
mmddyy = subject's birth date
(c) Participant location (ward) = CDC
(d) Birth date
(e) Sex

(5) The laboratory tests required for each study participant are entered by keying the number for the battery header tests. When the battery header is requested, the system orders the tests that are members of the battery.

(6) To assign a collection date to the sample, the laboratory assistant enters mmdd"D," with mmdd being the month and date the participant will have his laboratory tests performed.

(7) To request labels for the requested tests, the laboratory assistant enters the line number of the label printer, followed by an "L."

(8) After this information has been entered, the system summarizes the entry, assigns the sample number(s) and displays the requests on the CRT for review. When verified, the test requests are entered into the patient file and labels are printed.

b. Quality control (QC) test requests

(1) The laboratory system handles each control as a "participant." Each control material is assigned a participant number within a range of numbers specified by the LIS supervisor and is admitted to the "CDCQ" ward.

(2) The LIS automatically orders the desired test(s) for each control and assigns sample numbers for use each day.

(3) A list is generated showing all controls, the tests requested, and the assigned sample numbers.

c. "Blind" repeat test requests

(1) Five percent of all study specimens are repeated by "blind" analysis.

(2) Because of the large amount of blood required for testing, three randomly selected participants are used to make up a single complete battery of blind repeat tests. Each repeat sample is assigned a unique participant identification (ID) number, which can be linked through the LIS to the original number.

(3) The laboratory QC supervisor randomly assigns the tests to be requested on each of the three participants to ensure that all repeat tests are requested.

2. Sample Status

a. Sample collection verification

(1) The phlebotomist verifies blood collection and receipt of the random urine in the LIS, using the function "VSS."

(2) The laboratory assistants verify collection of the 12-hour urine specimens with the same function.

(3) To verify collection, the individual inputs "VSS," his/her technician code, the collection time, and the sample number of the specimen. If no collection time is specified, the LIS uses current system time. This information is entered into the paticipant file so that an audit trail can be maintained.

b. Worksheets

(1) For each instrument or manual procedure, the LIS generates a worksheet, which the technologist uses as a work-aid document.

(2) Worksheets contain the following information: sample number, date and time collected, participant name and ID number, test(s) requested, and a space for recording test results.

c. All QC sample numbers, names of control products, test(s) requested, and a space for recording results are also on the worksheet.

3. Data Entry and Verification Procedure

a. Automated noninterfaced instruments

(1) The technologist who performs the tests will input the results into the LIS after the run has been accepted.

(a) After results come off the instrument, record the values on the prepared worksheet, being careful to match the sample position on the run and worksheet.

(b) Manually enter the results for each participant into the LIS via a CRT.

(c) When all results are entered, review for accuracy and leave the results unverified for further review.

(d) Request the central facility (CF) to print a report for the results from a desired run and ask another technologist to verify the data entry.

(2) The verifying technologist will check results on the printed computer report against the instrument output document read aloud by the performing technologist.

(a) Carefully check (as it is read aloud) the result for each sample position on the instrument printout against the corresponding sample position on the printed report.

(b) If the results on the printed report agree with the instrument printout, initial the printed report, attach it to the handwritten worksheet and instrument printout, and return the set to the performing technologist. Data entry verification is complete.

(c) If the results on the printed report do NOT agree with the instrument printout, notify the performing technologist of the discrepancy.

(3) The technologist who performed the tests will determine the source of the discrepancy and make necessary corrections.

(a) Check the instrument output document against the worksheet to determine if the result was recorded incorrectly.

(b) Make necessary corrections on the worksheet if the result was written incorrectly. Initial and document any changes on the worksheet.

(c) Check the worksheet results against the results on the computer printed report to determine if all results were keyed correctly.

(d) Make necessary changes in the computer report via the CRT; initial and document any changes on the printed chart report.

(e) If no errors were discovered or changes made, notify the verifying technologist to recheck the results.

(f) If changes were made in the computer, request another printed report from the central facility and proceed through the verification checks again, starting at part 3a(1)(d).

(4) After all verification procedures are complete and no errors exist, the verifying technologist initials the chart report as reviewed and complete, attaches the worksheet to the instrument printout and notifies the performing technologist that data entry review is complete.

(5) The performing technologist confirms all results of the run in the LIS.

(a) Verify the run in the LIS via a CRT.

(b) Staple all paperwork for the run together and file for future reference.

b. Automated interfaced instruments

(1) The technologist who performed the run will review all results after the run has been accepted.

(a) After the run is complete, check each result in the computer with the result on the instrument printout.

(b) If results do not agree, notify the LIS supervisor of the possible interface malfunction.

(c) If results agree, request a printed report for the run from the central facility and ask another technologist to verify the data transfer.

(2) The verifying technologist will check results on the printed computer report against the instrument output document read aloud by the performing technologist.

(a) Carefully check (as it is read aloud) the result for each sample position on the instrument printout against the corresponding sample position on the printed report

(b) If the results of the printed report agree with the instrument printout, initial the printed report, attach it to the instrument printout, and return the set to the performing technologist. Data transfer verification is complete.

(c) If the results of the printed report, do NOT agree with the instrument printout, notify the performing technologist of the discrepancy.

(3) The performing technologist will determine the source of the discrepancy, and all verification steps will be repeated, starting at 3a(1)(a).

(4) After all verification procedures are complete and no errors exist, the verifying technologist initials the printed report as reviewed and complete, attaches it to the instrument printout, and notifies the performing technologist that the data transfer review is complete.

(5) The performing technologist confirms all results of the run in the LIS.

(a) Verify the run in the computer via the CRT.

(b) Staple all paperwork for the run together and file for future reference.

c. Manual tests

(1) The technologist who performs the test will input the results into the LIS after the run has been accepted.

(a) After all results are completed and recorded on the worksheet, manually enter the results for each participant into the LIS via a CRT.

(b) When all results are entered, review for accuracy and leave the results unverified for further review.

(c) Request the central facility (CF) to print a report for the run and ask another technologist to verify data entry.

(2) The verifying technologist will compare the worksheet results read aloud by the performing technologist with the printed computer report.

(a) Carefully check (as it is read aloud) the worksheet results against the printed computer results for each patient.

(b) If the results on the printed report agree with the worksheet results, initial the printed report, attach it

to the worksheet, and return the set to the performing technologist. Data entry verification is complete.

(c) If the results on the printed report do NOT agree with the worksheet results, notify the performing technologist of the discrepancy.

(3) The technologist who performed the test will determine the source of the discrepancy and make necessary corrections.

(a) Carefully check the worksheet results against the printed computer results for each patient.

(b) If no errors are discovered, notify verifying technologist to recheck the results.

(c) If an error is detected, make the necessary correction in the computer via a CRT and review for accuracy.

(d) Initial and document all changes on the printed computer report and request another printed report from the central facility.

(e) Proceed through the verification checks, again starting at 3a(1)(c).

d. Calculations

(1) The LIS performs many calculations using the verified results stored in the participant file. Formulas for calculations are located in a separate file in the LIS.

(2) Calculations are routinely performed for the following:

(a) 12-hour urine creatinine
(b) Absolute triglycerides
(c) Corrected glycerol blank
(d) Absolute T and B lymphocytes
(e) T4/T8 ratio
(f) Urine porphobilinogen
(g) D-glucaric acid
(h) FTI (free thyroxine index)

(3) The calculations are automatically performed by entering the test number followed by a "C," and the sample number. The verified results used in the calculation are displayed for review, and a prompt for the calculated results appears on the CRT. After "NN" and return are entered, the calculation is completed and displayed for review and verification by the technologist.

4. Participant Test Result Reviews

   a. Supervisor review

      (1) A report is generated at the end of the shift by the LIS for review by the QC supervisor.

      (2) This review includes the participant name, ID number, and age, and is set up to print all test results for each participant in a ward report format.

      (3) The supervisor reviews all results and ensures that all work is complete for each participant.

      (4) Abnormal values are flagged "H" or "L," and reference ranges are printed on this report.

      (5) All results, verified and unverified, are printed.

      (6) Since all QC data are handled in the same manner as "participant" data, QC results can also be printed on this type of a report and reviewed.

   b. Abnormal test results review

      (1) At the end of the shift, a report of all abnormal test results is printed for review by the supervisor.

      (2) The abnormal values printed are those that fall outside the reference ranges established for the study.

      (3) Participant name, ID number, sample number, test name, and test result are included on the report, with the results flagged "H" (for high) or "L" (for low).

   c. Incomplete list

      (1) At the end of the shift, a report of all incomplete tests is printed for review by the supervisor.

      (2) Incomplete tests are those that do not have verified results.

      (3) This list is used for follow-up to ensure that no tests are missing, that no results are left unverified, and that no answers to tests are missing.

5. Participant Hardcopy Reports

   a. On the afternoon of day 3 of the medical examination, a permanent participant results report is generated. This report includes all the tests requested and the status of each -- either a result or an incomplete message.

b. The printed reports are reviewed for completeness and internal consistency of results by the clinical pathologist. After review, they are ready for charting.

c. The medical records clerk picks up these reports and files them in the participants' records.

d. If any test results are outstanding at the time the permanent report is printed, the medical records clerk will be notified. She or he in turn will alert the diagnosticians.

e. When all test results are completed and entered into the LIS, a new permanent report is printed and given to medical records for filing in the participant's chart. The old, incomplete report is destroyed.

e. The medical records clerk will notify the medical director of the completion of these tests, and he or she will contact the participant to give him the test results.

6. Error Correction

It is anticipated that any and all errors will be detected and corrected before results are verified and stored in the LIS participant file. When a result is found to be incorrect and must be changed, the following procedure will be followed:

a. The QC supervisor will document the needed change on a request form and give it to the LIS supervisor or the QC supervisor will correct the result.

b. Only the LIS supervisor or the QC supervisor will be allowed to change a result in a participant's history file.

c. The LIS or QC supervisor, through the background function of the system, will remove the incorrect result and replace it with the correct result.

d. A new permanent participant report will be printed, reviewed by the clinical pathologist, and taken to the medical records clerk for filing in the participant's chart. The old participant report will be destroyed.

e. Arrangements will be made with CDC and the Clinic Data Management Section to retransmit the correct laboratory data for that participant.

f. Accurate documentation of the action will be maintained in the LIS central facility. Documentation will include participant name, ID number, test name, previous incorrect result, new corrected result, time and date the correction was made, and the signature of the LIS or QC supervisor.

7. Transfer of Data to CDC

a. Participant data

Monthly, the laboratory participant data are downloaded to a magnetic tape in the central facility of the mainframe computer. These tapes are then sent to the Centers for Disease Control in Atlanta, Georgia. CDC will perform automated edit checks for record completeness and for validity and consistency of data. Any edit rejections will be listed and sent back to the QC supervisor for correction or verification.

b. Quality control data

The laboratory QC supervisor has an IBM-PC-XT connected to the CHC system with a terminal emulator package. Weekly, quality control data are downloaded from the CHC system and formatted by using a software program. The QC supervisor creates a data file of that week's control data and transmits these data to CDC via Crosstalk for CDC's evaluation and review.

III. SPECIMEN COLLECTION

A. Specimen Collection Packet Preparation

1. Principle

This procedure is used to prepare packets of collection bottles and tubes for blood and urine specimens obtained from each participant. Computer-generated labels are applied with appropriate participant and assay identification to each collection container and then organized in a packet for each participant. Some additional tubes are prepared for additional collections for blind repeat laboratory determinations.

2. Supplies and Equipment

a. Sodium carbonate
b. Labels identifying the preservative
c. Computer name labels
d. Semen cup labels
e. Log sheets
f. Ziploc bags
g. 5-oz urine collection cups
h. 2-oz specimen collection cups
i. Serum separation Vacutainer tubes--15 ml
j. Sodium citrate anticoagulated Vacutainer tubes--5 ml
k. Sodium heparin anticoagulated Vacutainer tubes--7 ml
l. Sodium heparin anticoagulated Vacutainer tubes--3 ml
m. Ethylenediamine tetra-acetate anticoagulated Vacutainer tubes--7 ml
n. 6-oz Styrofoam paper cup with lid
o. Paper bags--size #10

3. Procedure

a. 12-hour urine collection packet

(1) From the participant list provided, order the battery headers for the tests to be performed on the 12-hour urine collection. Be sure to include the correct collection day in your entry string.

(2) Generate the computer labels.

(3) Obtain a urine collection bottle containing the appropriate preservative (2.0 g Na carbonate).

(4) Prepare a large adhesive urine container label with the appropriate identification information and preservative and affix it to the side of the urine collection bottle.

(5) Also affix the computer (tube) label with the individual sample number, participant's identification, requested tests, and date to the side of the bottle.

(6) On a separate, blank computer label, record the participant's full name, identification number, and the date the collection is to be started and affix this label to the urine container.

(7) Retain the computer-generated aliquot labels in specimen processing for use when the specimen is submitted.

(8) Prepare a 12-hour urine log sheet for each day that specimens will be submitted. Ensure that participant's identification and sample numbers match the collection packets prepared.

b. Fasting blood collection packet

(1) From the participant list provided, order the battery header for the tests to be performed. Be sure to indicate the correct date of collection in your entry string.

(2) Generate the computer labels.

(3) Obtain the evacuated tubes necessary for the specimen collection and affix the appropriate computer (tube) labels to the sides of the tubes.

(a) Seven 15 ml SST tubes (serum separation tubes)
(b) One 7 ml Na heparin (green-top) tube (sodium heparin)
(c) Two 7 ml EDTA (purple-top) tube (ethylenediamine tetra-acetate)
(d) One 3 ml Na heparin (green-top) tube
(e) One 5 ml Na citrate (blue top) tube (sodium citrate)

(4) Place the labeled evacuated tubes and all the computer aliquot labels into a Ziploc bag.

(5) Label the outside of the packet with COMPLETE participant information (full name, identification number, and date of collection).

(6) Prepare a fasting blood specimen log sheet for each date of collection. Ensure that participant's identification and sample numbers match the labels on the evacuated tubes in the collection packet.

c. Random urine collection packet

(1) From the participant list provided, order the battery header for the random urinalysis. Be sure to indicate the correct date of collection in your entry string.

(2) Generate the computer labels.

(3) Obtain an all-purpose specimen collection container and affix the computer (tube) label to the side of the container.

(4) Retain the remaining computer-generated aliquot labels in specimen processing for use when the specimens are submitted.

(5) Place the labeled urine collection container into the appropriate fasting blood collection packet, being very careful to match participant identification numbers and dates of collection.

d. Semen collection packet

(1) Place a preprinted label on a specimen container.
(2) Place the specimen container and a Styrofoam cup with a lid in a paper bag for delivery to the participants.

e. Blind sample collection component

(1) From the participant list provided, order the appropriate blind repeat tests on the participants designated.

(2) Generate labels and add to packets (except 12-hour UA cups, which will be aliquoted in the processing area):

For Blind "A"--12-hour urine		3 UA cups
	Random urinalysis	1 UA cup
	Hepatitis screen	1-7 ml SST
	Chemistry profile	1-7 ml SST
For Blind "B"--RIA testing		1-7 ml SST
	ICS testing	1-7 ml SST
For Blind "C"--Hematology testing		1-5 ml EDTA
	T and B testing	1-7 ml Na Hep
	Protime testing	1-5 ml Sodium Citrate

(3) The combination of blinds A, B, and C will equal one participant. Once the blood is drawn from the original participant, the blind tubes are separated from the other tubes by the processer and treated as a separate participant sample.

III. SPECIMEN COLLECTION

B. Blood Collection

1. Principle

This procedure establishes criteria for the proper collection of blood specimens by venipuncture. Blood drawn by laboratory phlebotomists will be obtained ONLY from veins in the arm.

2. Supplies and Equipment

a. Venipuncture couches
b. Needles (21-gauge preferred)
c. Sterile syringes
d. Vacutainers
e. Evacuated tubes
f. Blood pressure cuffs
g. Alcohol swabs
h. Gauze pads or cotton balls
i. Needle destroyer and disposal boxes
j. Adhesive bandages
k. 5% bleach solution

3. Procedure

a. Table preparation:

(1) Obtain specimen collection packet identified for the participant whose blood is to be drawn.

(2) Remove all evacuated tubes and place on utility table for easy access during venipuncture procedure.

(3) Check tube labels and verify that the identification number and birth date correspond with the participant information on the outside of the packet and that all tubes have the same assigned sample number.

b. Participant identification:

(1) Ask the participant to give his full name and birth date.

(2) Compare this information with the information on the participant's identification bracelet and the information on the collection packet.

(3) Have the participant sign in on the phlebotomy log sheet opposite his name.

c. Participant preparation verification:

(1) Ask the participant if he is fasting and note the response on the questionnaire.

(2) Ask the participant for the preparation history questionnaire. (This may have already been collected by the participant advocate.)

d. Participant reassurance:

(1) If the person seems anxious, take some time to explain what is to be expected.

(2) It is wise to tell the person when the needle enters the skin so that he will not be frightened.

(3) Never tell a participant, "This won't hurt."

e. Positioning the participant:

(1) Be sure the participant is seated well back in the chair and is comfortable.

(2) The arm should be supported firmly by the arm rest and extended to form a straignt line from the shoulder to the wrist.

f. Assembling supplies:

(1) Assemble the following:

(a) Labeled collection tubes
(b) Blood pressure cuff
(c) Alcohol swabs
(d) Gauze pads or cotton balls (cotton balls should be used for participants with dermatitis)

(2) Select the system for drawing the blood specimen.

(a) An evacuated system is preferred.

(b) Plastic syringes may be used when blood is being drawn from persons with fragile, thready, or "rolly" vein walls.

g. Selecting the vein site:

(1) The median cubital and cephalic veins are preferred. Wrist and hand veins are also acceptable for venipuncture.

(2) With the index finger, palpate and trace the path of the veins several times.

(3) If superficial veins are not readily apparent, you may:

(a) Apply a blood pressure cuff briefly.
(b) Massage the arm from wrist to elbow.
(c) Tap at the vein site sharply with the index and second finger.
(d) Apply a warm, damp washcloth (40 ^{o}C) to proposed site.
(e) Lower the arm over the bedside or venipuncture chair.

h. Cleansing the venipuncture site:

(1) Remove the prepared alcohol pad from its sterile package.

(2) Cleanse the vein site with a circular motion from the center to the periphery.

(3) Allow the area to dry.

(4) If the vein site must be touched again, it should be cleansed again.

i. Applying the blood pressure cuff:

(1) Never leave the blood pressure cuff inflated for longer than 1 minute.

(2) If you must apply a blood pressure cuff for preliminary vein selection, release it and after waiting 2 minutes, reapply it.

(3) Wrap the blood pressure cuff around the arm 3 to 4 inches above the venipuncture site. Stick the Velcro tabs on the blood pressure cuff to each other.

j. Inspecting the needle and syringe:

(1) The appropriate needle is attached to the Vacutainer or syringe.

(2) When it is time to use the needle, it should be removed from the sheath and examined visually to make sure that it is free of hooks at the end of the point, and that the opening is clear of small particles.

(3) If a syringe is being used, move the plunger within the barrel to show that the syringe and needle are patent and that the plunger is moving freely.

k. Performing the venipuncture:

(1) Evacuated tubes

(a) Collect the tubes in the same order every time. See procedural notes.

(b) Insert the blood collection tube into the Vacutainer and into the needle up to the recessed guideline on the needle holder. To prevent the loss of vacuum, do not push the tube beyond the guideline.

(c) Grasp the participant's arm firmly, using the thumb to draw the skin taut, thus anchoring the vein.

(d) With the bevel up, line up the needle with the vein. Push the needle into the vein. Hold the needle holder with one hand and with the other hand, depress the tube forward until the butt end of the needle punctures the stopper and activates the vacuum action. When the needle is in the vein, keep the tube below the site.

(e) Release the pressure from the blood pressure cuff as soon as the blood flow is established.

(f) Fill the tube until the vacuum is exhausted and the blood flow ceases.

(g) When the blood flow ceases, remove the tube from the holder. The shut-off valve recovers the point, stopping the blood flow until the next tube is inserted.

(h) Immediately after drawing, the blood, mix the contents of each tube that contains an anticoagulant by gently inverting the tube 5 to 10 times.

(i) To obtain additional specimens, insert the next tube into the holder and repeat the procedure from step (f)

(2) Butterfly technique for difficult draws. (This technique requires an assistant.)

(a) Insert the needle of the butterfly into the vein, allowing the tubing to fill by capillary action.

(b) If necessary, tape the needle to the arm to prevent it from moving.

(c) Place the first syringe on the end of the tubing and, to fill it, gently pull the plunger.

(d) Bend the tubing over the plastic cap near the syringe to stop the blood flow. Remove the full syringe and hand it to the assistant. Place a clean syringe on the end release the tubing to continue the blood flow, and proceed as in step (c).

(e) When the appropriate amount of blood is obtained, remove the butterfly and treat the venipuncture with a cotton pad and pressure, as in a "normal" draw.

(f) Treatment of svringe-drawn specimens:

If evacuated tubes are filled from a syringe, stoppers should NOT be removed. The diaphragm of the rubber stopper on the appropriate tube MUST BE PUNCTURED and the correct amount of blood must be allowed to flow slowly into the tube. Blood should NEVER be forced into a tube. If the tube does not fill, the plunger of the syringe may be pushed gently (an extremely important technique).

l. Removal of the needle:

(1) Lightly place a gauze pad over the needle in the venipuncture site.

(2) Apply slight pressure to the pad and remove the needle slowly, while keeping the bevel in an upward position.

(3) Apply mild pressure to the venipuncture site until the bleeding stops.

(4) Apply an adhesive bandage over the venipuncture site and tell the participant to leave the bandage in place for at least 15 minutes.

m. Disposal of puncture unit:

(1) Clip the needle in the needle cutter, unscrew the hub from the holder or syringe, and drop it into the box.

(2) DO NOT throw the needle into a wastebasket.

n. Collection verification:

(1) Verify collection in the computer, using the VSS function.
(2) Initial the log sheet and record the time of the venipuncture.

o. Specimen handling:

(1) Deliver all blood specimens and the packet containing remaining labels to the preliminary processing site in the phlebotomy area.

(2) Wash hands thoroughly before going to the next participant.

4. Procedural Notes

a. Difficulties in drawing blood

(1) If blood cannot be obtained after two venipuncture attempts, the clinical manager or other designated nurse will be contacted to acquire the blood specimen

b. Order of draw for multiple specimens

(1) Nonadditive tubes (e.g., red stopper)
(2) Additive tubes in the following order:

(a) Sodium citrate (blue stopper)
(b) Sodium heparin (green stopper)
(c) Ethylenediamine tetra-acetate (purple stopper)

c. Prevention of hematomas (bruises)

(1) Do not go THROUGH the vein, only IN to it.

(2) Release blood pressure cuff pressure before removing the needle.

(3) Apply sufficient pressure so that bleeding has stopped before bandaging the venipuncture and dismissing the participant.

d. Prevention of hemolyzed specimens

(1) Mix anticoagulated specimens by gently inverting the tubes, not by shaking them.

(2) Avoid drawing the plunger back too forcefully when using a needle and syringe.

(3) Avoid using a needle that is too small. (A 21-gauge needle is preferred.)

e. Blood spills or contamination

(1) Blood spills or contaminated tubes and equipment are to be cleaned up and disinfected by using a 5% bleach solution.

f. Participant problems

(1) If a participant feels faint, lower the lounge back and administer an ammonia inhalant; if necessary, apply cold compresses to the forehead and back of the neck. If the participant does not respond, notify the clinical manager or physician.

(2) If a participant becomes nauseous, make him as comfortable as possible and instruct him to breathe deeply and slowly. Apply cold compresses to the forehead.

(3) If a participant suffers convulsions, prevent him from injuring himself but do not restrain the movements of the extremities completely. Call the clinical manager or the physician.

5. References

a. Koebke J, McFarland E, Mein M, Winkler B, Slockbower JM. Collection of blood specimens and venipuncture procedures. In: Slockbower JM, Blumenfeld TA, eds. Collection and handling of laboratory specimens, a practical guide. Philadelphia: J.P. Lippincott, 1983:3-33.

b. Slockbower JM, Belgeri KM, Bruck E, et al. Procedure for the collection of diagnostic blood specimens by venipuncture. NCCLS, 1984; 4(No.5).

III. SPECIMEN COLLECTION

C. Random Urine Collection

1. Principle

This procedure establishes criteria for the proper collection of random urine specimens for routine urinalysis.

2. Supplies and Equipment

a. 5-oz prelabeled urine collection cup (to be placed in the participant collection packet) (Sage catalog no. 2200).

3. Procedure

a. Obtain the specimen collection packet identified for the participant.

b. Ask the participant to give his full name and birth date and compare this information with the information on the participant's identification bracelet and information on the collection packet.

c. Remove the prelabeled specimen containers from the packet and verify that the participant's identification and sample number match the remaining labels and the information on the outside of the collection packet.

d. Instruct the participant to collect the urine specimen and place it in the designated basket in the phlebotomy room.

e. Record the time of collection on the log sheet and initial the entry. Verify the collection in the computer by using the VSS function.

f. Store the urine sample in the refrigerator (2-8 ^{o}C) until it is to be transported.

g. Specimens, computer labels, and log sheet are to be transported to specimen processing or directly to the research area within 1 hour from the time of collection.

4. Reference

Simindinger J, Mansour FK, Slockbower JM. Collection of nonblood specimens, specimens for urinalysis. In: Slockbower JM, Blumenfeld TA, eds. Collection and handling of laboratory specimens, a practical guide. Philadelphia: JP Lippincott, 1983:103.

III. SPECIMEN COLLECTION

D. 12-Hour Urine Specimen Collection

1. Principle

This section describes instructions to be given to the participant regarding the method of collecting of a 12-hour fasting urine specimen.

2. Supplies and Equipment

a. 4-pt container (Texberry catalog no. 48009) with
b. 89 mm lid (Texberry catalog no. 30548)
c. Igloo Playmate cooler - 10-quart size
d. Frozen ice pack, 4" x 6", placed in each cooler

3. Instructions (to the participant)

a. You are supplied with the proper container with preservatives added to ensure the stability of the analytes for the study. To further protect the specimen, we ask that the urine container be returned to the specimen cooler and covered between collections. Moderate intake of water is allowed during the collection period.

b. All urine voided during a 12-hour period is required.

(1) At 7 p.m. on the evening of starting the urine collection empty the bladder and DISCARD this urine. Record the DATE and TIME this was done on the container label after the word "start."

(2) Collect ALL urine during the next 12 hours until 7 a.m. the following morning.

(3) At this time, empty the bladder and ADD this urine into the 12-hour collection of the urine. Record the DATE and TIME on the container label after the word "end."

c. Following the above plan assures that the bladder is empty before the start of the collection and that it is empty upon the completion of the collection.

IN CASE OF ACCIDENTAL INGESTION, YOU SHOULD KNOW THAT THE PRESERVATIVE IN THE URINE CONTAINER IS SODIUM CARBONATE. CONSULT A PHYSICIAN.

III. SPECIMEN COLLECTION

E. Semen Specimen Collection

1. Principle

This section describes instructions to be given to the participant regarding method of collection of a semen specimen. Specimens are to be collected in the participant's hotel room.

2. Supplies and Equipment

a. 2-oz prelabeled specimen collection cup (Sage catalog no. 2210)
b. 6-oz Styrofoam cup with lid
c. Paper bag (Toreodore catalog no. 10)

3. Instructions (to the participant)

a. Please DO NOT have sexual intercourse, masturbate, or engage in any other sexual activity that results in ejaculation for 2 days (36-48 hours) before submitting a semen sample for evaluation in this study. The number of days of abstinence (days without sex) will affect the number of sperm in your sample.

b. After the necessary period of abstinence, produce a semen sample by masturbation and collect the entire amount in the plastic container provided. DON'T use a condom (sheath) or any other method. Place the lid securely on the container when the semen collection is complete.

c. Write on the label when you collected the sample (date, time) and indicate whether any missed the jar or was spilled. Also note length of abstinence and whether a vasectomy has been performed.

d. When all the label information is complete, place the container into the Styrofoam cup, cap tightly, and bring the cap to the specimen processing room as soon as possible after you collect the specimen, preferably within 30 minutes.

IV. SPECIMEN HANDLING AND PROCESSING

A. Blood Specimen Handling and Processing

1. Principle

This procedure establishes criteria for optimal samples and addresses the handling and processing of blood specimens for analytical determinations and for shipment of additional samples to the Centers for Disease Control for long-term storage.

2. Equipment

a. Refrigerated centrifuge
b. Cold block
c. Polypropylene tubes
d. Glass culture tubes
e. Tube closure
f. Plastic transfer pipettes
g. Wheaton vials, 5 ml
h. Refrigerator
i Freezer
j. Adjustable macro MLA pipette
k. Wheaton rubber stopper
l. Wheaton aluminum seals
m. Dry ice - 100 lb/wk

3. Procedure at Blood Collection Site

a. Specimen handling

(1) Place the blue top (coagulation) tubes immediately into the cold block and maintain at 2-8 °C.
(2) Properly preserve the blood specimen for T & B cells into the labeled sodium heparin tube (approximately 5 ml) stopper and invert the tube gently to mix the contents. Maintain in separate rack in upright position at room temperature until transport.
(3) Place all SST tubes into another rack and allow to clot undisturbed at room temperature.
(4) Place all EDTA tubes for hematology profiles into a separate rack.
(5) Sort the computer labels and match with specimens.
(6) Place the labels into separate envelopes to accompany each rack of specimen tubes.

b. Plasma aliquots

(1) Within 30 minutes after venipuncture, centrifuge the blue top (coagulation) tubes at 4 °C at 1,000-1,200 X g for 10 minutes.

(2) While the blood tubes are centrifuging, label polypropylene tubes with the appropriate aliquot labels and place these tubes in a rack.
(3) On the edge of the rack in front of each tube, place an extra computer label making sure it matches the identification and sample numbers on the tube label.
(4) Carefully check and match identification and sample numbers on spun blue top tubes and aliquot tubes and transfer pipet. Plasma must be free of excessive turbidity, particulate matter, and visible hemolysis.
(5) Place the extra label from the edge of the rack onto a log sheet when aliquoting is complete for each sample and initial the sheet. This technique reduces the error of mismatching.
(6) Tightly cap the aliquot tubes and maintain at 2-8 °C in the cold block until transport.
(7) Dispose of original tube containing the red cells into disposal cans for autoclaving.

c. Transport all blood specimens, the envelopes containing the aliquot labels, and the specimen transport log sheet within 45 minutes from time of collection. Include in each cooler a minimum-maximum thermometer that has been reset and is currently at 2-8 °C.

4. Procedure at Laboratory Processing Area

a. Receiving and specimen handling

(1) Initial the specimen transport log sheet and indicate the time the specimens arrived in the processing area. Also record the minimum and maximum temperature of the cooler.
(2) Specimens that do not require processing may be routed directly to the testing area, i.e., hematology profiles and plasma aliquots for prothrombin time. Initial and note time of distribution on specimen acceptance log.
(3) Place the SST tubes into the centrifuge in such a way that all specimens are balanced. Be sure stoppers remain in place for centrifugation. Spin at 1,000-1,200 X g for 10 minutes at room temperature (22-25 °C.).

b. Serum aliquots for analysis

(1) Before receipt of patient specimens in the processing area, the following should be prepared:

(a) Specimen processing checklist with the day's particpants and blind sample test requests entered.
(b) Specimen processing sample log with labels affixed for each participant (this log will be used for preparation of the CDC aliquots in Section 4).
(c) A rack containing capped, labelled aliquot tubes for delta-ALA and melioidosis.

(d) Three racks labeled as follows:

RIA
Ektachem
Hepatitis

(2) Remove the SST tubes from the centrifuge and group them by sample numbers in a test tube rack. Working with one patient set of tubes at a time, place one tube each in the labeled racks for RIA (radioimmunoassay), Ektachem, and hepatitis. One tube will also go to the aliquot rack for D-ALA and one to the aliquot rack for melioidosis.

The D-ALA tube will receive 3.5 ml of serum. The melioidosis tube will receive 1.5 ml of serum.

Extra tubes will go in another rack to be stored at 2-8 ^{o}C for later aliquoting for CDC specimens.

c. Distribution and storage of aliquots

(1) Notify the testing areas when samples are ready for analysis.
(2) Store the aliquots appropriately (refer to storage instructions in card file) until all participant samples have been processed.
(3) Have the receiving technologist note the time of receipt and initial the Specimen Acceptance Log. (If a courier takes specimens to laboratory areas or the research building, make sure that the technician's initials and time received by the performing technician are also recorded on the Specimen Acceptance Log.)

d. Spare serum specimens

For each participant:

(1) Appropriately label three Wheaton vials containing the spare serum specimens for shipment to CDC. Follow the CDC protocol for identification scheme. (Section 5.) Record the CDC ID number on the Specimen Processing log adjacent to our Community Health Computer (CHC) generated label.
(2) Affix CHC-generated labels to one additional Wheaton vial to be utilized as a spare serum specimen by our laboratory (vial D).
(3) Upon return of the SST tubes used for RIA, chemistry, and hepatitis testing, carefully match the specimens to the spare SST tubes that have been stored in the processing refrigerator.
(4) Working from the Specimen Processing Log, match the labelled SST tubes to the four labeled Wheaton vials.

(5) With the adjustable MLA pipette, measure 3.3 ml of serum into each of the Wheaton vials. It is important that the three vials to be shipped to CDC are aliquoted first.
(6) If any specimens are insufficient for all three CDC Wheaton vials, aliquot the short amount into vial C. Vials A and B should contain 3.3 ml each.
(7) Stopper the serum bottles and put seal in place, using the air-powered crimper.
(8) Place bottle into the crimping jaws and depress foot valve. Hold until bottle is sealed. Release foot valve and remove sealed bottle. Check seal for tightness and appearance.
(9) Store the sealed vials in freezer at -20 ^{o}C.
(10) Record time of storage and initial the log sheet. Record on the log sheet the number of vials stored for CDC and our laboratory as well as the box number the vials were stored in.

5. Protocol for Shipment of Spare Serum Specimens

a. Supplies needed:

(1) Wheaton 5 ml serum bottle-clear
CMS no. 026-306 288/case
Keep inventory stocked to 30-60 cases (do not let our supply drop below 20 cases)
(2) Wheaton rubber stopper, gray butyl
CMS no. 026-443 1,000/case (at 5 cases-order to 10)
(3) Wheaton tear-off aluminum seal
CMS no. 131-698 1,000/case (at 5 cases-order to 10)
(4) CDC aliquot bottle label
Time products DPC-8 (quote no. 2137 for specs)
(5) Adjustable MLA pipet (1-5 ml)
(6) MLA pipet tips Macro-9048
CMS 396-077 100/pkg

b. Identification number for each participant specimen:
AO-XXXXX-Z

Where: AO = Agent Orange Projects identifier
XXXXX = Participant ID number starting with 00001 and continuing consecutively
Z = Specimen aliquot numbers for each participant (A, B, C, and D)

c. Instructions for completing "Specimen Shipping List"

Each "Specimen Shipping List" can accommodate participant ID information for 2 specimen boxes with 12 participants/box.

(1) Enter 3-digit ID number for Box A as described in Section d below.
(2) Enter 5-digit participant ID numbers for 12 participants. If only 1 or 2 specimens are shipped for a participant, indicate the number in the "No. Specimens" column. If a completed set of 3 specimens is shipped, then no entry is necessary in this column.

IMPORTANT: 12 PARTICIPANT SPECIMENS MUST BE INCLUDED IN EACH BOX.

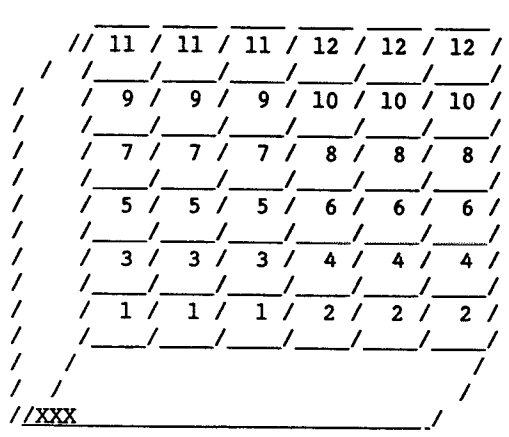
(3) Repeat steps (1) and (2) above for Box-B.
(4) After the "Specimen Shipping List" is completed for Boxes A and B and the specimens are ready for shipment to CDC, then complete the "Shipped By" line.

d. Instructions for labeling "Specimen Boxes"

Each 36-cell specimen box mailed to CDC must be labeled with a unique 3-digit ID number. The study's first specimen box should be labeled 001 with each subsequent box being numbered consecutively for the entire study.

A notebook containing a copy of all specimen shipping lists should be maintained at the contract laboratory to avoid duplicating or omitting box numbers. Using a ballpoint pen, write the number on the box top (at a corner) identifying the location of Box Position number 1. Write the box number in a position sufficiently low so that the box top will not cover the number. See diagram of specimen box.

Diagram of Specimen Box



Top View

XXX = Specimen box ID number
1-12 = Specimen box position number for each set of participant samples

e. Instructions for packing specimen boxes:

(1) For each participant a set of three frozen serum specimens (A, B, and C) will be shipped to the CDC.

(2) Specimens A, B, and C are to be positioned in the box as shown in the "Diagram of Specimen Box" (shown above) using the box position number from the "Specimen Shipping List." If a participant has less than three specimens leave the allocated space empty. Specimens are packed left to right, starting in the lower left corner using the box number as a starting point.

f. Shipment of specimens to CDC:

(1) Specimens are to be shipped to the CDC only on Tuesdays.

IMPORTANT: Since the materials packed in accordance with the instructions below will remain frozen only about 2 1/2 days, shipments should not arrive in Atlanta on weekends or on Federal holidays.

(2) For all shipments, do not pack the shippers with frozen specimens and dry ice until just before transport to the Express Mail office at Albuquerque International Airport.

(3) Dry ice is delivered every Monday by Argyle Welding Supply, Inc., Albuquerque, New Mexico (Telephone number (505) 345-8101. (Specify 100 lbs dry ice, 1- by 7- by 10-inch slabs).

Note: When packing the shippers, wear gloves when handling the dry ice to avoid burning the hands. Glasses or an eye shield should also be worn if the dry ice cakes are to be broken into small pieces.

(4) Instructions for packing and shipping frozen serum specimen vials A, B, and C to CDC.

Remember: Mail only totally filled shippers that contain specimens from 24 participants in two-specimen boxes. Any number of participant specimens not divisible by 24 must be stored at the contract laboratory until enough specimens to fill a full shipper are available for shipment.

(a) Seal each specimen box with filament tape.

(b) Pack four specimen boxes (Boxes A and B from two shipping lists) per Styrofoam shipper.

(c) Fill the shipper with dry ice (it probably will hold 10-12 lbs) and place the Styrofoam lid on top of the shipper.

(d) Secure the completed "Specimen Shipping List" in a Ziploc bag and attach to the top of the Styrofoam lid with filament tape. In addition, mail a photocopy copy of the "Specimen Shipping List" in a separate envelope to the same CDC address (see (f)(i) below). This is to ensure against loss and considerable time lag in detecting missing shippers. Secure the outer carton lid on the shipper with filament tape.

(e) Cover or remove previous labels on all shippers.

(f) Additional Express Mail labels are available at the Post Office located in the Clinical Pharmacy on Gibson Street.

(i) Preaddressed, franked CDC mailing label with following address:

Brenda Lewis
Centers for Disease Control
Chamblee Bldg. 31 Rm. 8
Atlanta, GA 30333

(ii) Express Mail label with the same CDC address as above typed in.

(iii) DRY ICE label, with the weight of dry ice added.

(iv) Dry ice, form with weight of dry ice, shipper's address, and CDC address.

(v) HUMAN BLOOD label.

(vi) Call the courier to pick up the shippers by 11:30 a.m. He or she is to deliver the shippers to the Express Mail drop at Albuquerque International Airport by 12 p.m. on Tuesday for delivery to CDC within 24 hours.

(vii) Telephone the laboratory at CDC the day the shipment is mailed.

(5) Shipping Supplies - Contact the laboratory at CDC for requesting supplies a-d
   (a) Specimen boxes, 6- by 6- by 2-inches high, 36 cell
   (b) Styrofoam shippers
   (c) Filament tape for securing shippers
   (d) Prenumbered labels for the aliquot bottles
   (e) Preaddressed franked mailing labels
   (f) Dry ice labels
   (g) Transparent tape for securing specimen boxes
   (h) Specimen shipping list
   (i) Protocol
   (j) Ziploc bags
   (k) Human blood labels containing after-hours delivery instructions

6. Procedure Notes

   a. Criteria for specimen rejection:

      (1) Inadequate specimen identification.

      (2) Inadequate volume of blood collected into an additive tube.

      (3) Excessive hemolysis of specimen.

      (4) Improper transportation of samples.

7. References

   a. Calam RR, Benoit SW, DuBois JA, et al. Procedures for the handling and processing of blood specimens. NCCLS, 1984;4(No.9).

   b. Mansour FK. Processing of specimens. In: Slockbower JM, Blumenfeld TA, eds. Collection and handling of laboratory specimens, a practical guide. Philadelphia: Lippincott, 1983:172-91.

IV. SPECIMEN HANDLING AND PROCESSING

B. Urine Specimen Handling and Processing

1. Principle

This procedure establishes criteria for handling and processing urine specimens to ensure optimal samples for analytical testing.

2. Equipment

a. Top loading balance
b. 100-ml. specimen containers with screw-on lids
c. Preweighed 0.05-g aliquots of Na_4EDTA (tetra-sodium ethylenediamine tetra-acetate). (SIGMA, order no. ED455.)
d. 50 ml Wheaton vials and stoppers

3. Procedure for 12-Hour Urine Specimen Processing

a. Receiving and specimen handling

(1) Upon receipt of the 12-hour specimens, denote time, and initial the log sheet. Ensure that the specimen identification numbers and sample numbers match those on the log sheet. Record the preweight of the container with preservative. This weight is recorded on the urine container and was measured before the container was delivered to the participant.

(2) Weigh each 12-hour specimen and record weight (to the nearest tenth of a gram) on the container and on the log sheet, carefully checking identification and sample numbers on container and log sheet.

(3) Store specimens in the refrigerator (2-8 °C) until aliquoting can be done.

(4) Assume a constant specific gravity of 1.012 for each 12-hour urine collection and calculate the total volume (TV) for each specimen.

$$TV = \frac{\text{weight of specimen}}{1.012}$$

(5) Record the total volume on the log sheet. (Report TV to the nearest whole milliliter)

b. Aliquoting

(1) Affix computer aliquot labels to the appropriate aliquot containers.

(a) porphyrins - 100-ml specimen container
(b) porphobilinogen (PBG) and D-glucaric acid (D-gluc) - 100-ml specimen container
(c) creatinine - 100-ml specimen container
(d) storage aliquot - 50-ml Wheaton vial

(2) Remove corresponding 12-hour specimens from the refrigerator (do NOT process more than four specimens at one time).

(3) Carefully match specimens and aliquot containers by checking participant identification number and sample number.

(4) Mix specimen THOROUGHLY before aliquoting.

(5) Pour urine from the collection container into the appropriate container:

(a) Porphyrins = 50 ml
(b) PBG and D-gluc = 10 ml minimum
(c) Creatinine = 2 ml minimum
(d) Storage = 40-45 ml (if available)

(6) To each porphyrin aliquot (50 ml), add 0.05 g tetrasodium EDTA. Cap and invert several times to mix.

(7) Cap each aliquot container and store in the designated areas of the refrigerator until samples are distributed.

(8) The Wheaton vials are for stored specimens and are to be frozen at -20 °C.

(9) Enter the 12-hour urine total volume in the CHC computer, and verify the urine collection.

c. Distribution

(1) Notify each testing area when all participant specimens are aliquoted and ready for analysis. Store the urine aliquots at 2-8 °C if distribution to testing areas is delayed.

(2) On specimen acceptance log sheet, initial and record the time the samples are distributed for analysis.

(3) Be sure appropriate computer labels accompany aliquots.

(4) Record your initials on the log sheet when each specimen is completed and stored.

4. Random Urine Specimen Processing

   a. Receiving and handling

      (1) On the urine log sheet, record time specimens are received in processing and initial. Check to verify that all specimens are accounted for and that aliquot labels have accompanied the specimens.

      (2) Refrigerate all specimens at 2-8 °C until they are distributed to test area. No processing or aliquoting is to be done on the random samples.

   b. Distribution

      (1) Notify test area when all specimens are ready for analysis.

      (2) On the specimen acceptance log sheet, record the time the samples are distributed. Initial.

5. References

   a. Bermes EW, Forman DT. Basic laboratory principles and procedures. In: Tietz NW, ed. Fundamentals of clinical chemistry. Philadelphia: WB Saunders, 1976:52-3.

   b. Slockbower JM, Blumenfeld TA. Collection and handling of laboratory specimens. Philadelphia: Lippincott, 1983.

IV. SPECIMEN HANDLING AND PROCESSING

C. Semen Specimen Handling and Processing

1. Principle

This procedure describes the processing of semen specimens in preparation for automated semen analysis. Semen specimens are dropped in a mail-type holding bin outside the processing room (in the hotel where the samples are collected) by the participant either in the morning or afternoon designated collection times. A technologist is present in the processing room to receive and begin processing the sample. Selected semen characteristics are noted at this time.

2. Equipment

a. pH Meter-Orion 701A
b. Incubation oven (30 °C)
c. Vortex (Thermolyne Mix Max)
d. Slide staining rack
e. Microscope slides-frosted end
f. Pasteur pipets - 5-3/4 inch
g. 5-cc syringes
h. 19-Gauge syringe needles
i. pH Paper, 0-14 unit range
j. Buffer, pH 7
k. Buffer, pH 10
l. 95% Ethanol

3. Procedure

a. Fill out a specimen collection worksheet with participant's ID number, name, days of abstinence, collection time, vasectomy status, and time of specimen arrival at the processing room.

b. Keep specimen at 30 °C until ready for analysis. Specimen is ready for analysis 30 minutes after ejaculation.

Note: All processing, including videotaping of the specimen, should be completed within 2 hours of semen collection.

c. Vortex specimen in the collection container for 15 seconds WITH THE LID ON. Allow specimen to sit for a few seconds after vortexing to allow aerosols to dissipate before the container is opened.

d. Classify the color of the semen:

(1) Normal (gray-white, translucent)
(2) Brownish, reddish, yellowish, or white (abnormal)

e. Classify the odor of the semen. Do not purposely smell the specimen. If a specimen is obviously not normal (musky smelling), it will be obvious when the lid is removed. Classify as:

(1) Normal
(2) Abnormal

f. Measure the volume of the semen. Use a 5-cc syringe with an 18-19-gauge needle. Measure to the nearest 0.1 ml. Dispense back into the container.

g. Measure the pH of the semen to the nearest 0.1 unit.

h. Measure the viscosity of the semen 30 minutes after ejaculation (after measuring the volume). Aspirate a small amount of seminal fluid into a transfer pipet. Slowly expel and grade as following:

(1) Normal
(2) Minor, moderate, or severe (abnormal).

i. Before placing semen in the Mackler Chamber (in preparation for videotaping the specimen for automated semen analysis), swirl the container to assure homogeneity of the sample.

4. References

a. Cannon DC. Seminal fluid. In: Henry JB, ed. Clinical diagnosis and management by laboratory methods. Philadelphia: WB Saunders, 1984:516-9.

b. Schrader SM. Semen analysis. Cincinnati: National Institute for Occupational Safety and Health, 1983; SOP no. EA-60(1).

c. Urry R. Seminal fluid. In: Kjeldsberg CR, Knight JA, eds. Body fluids, laboratory examination of amniotic, cerebrospinal, seminal, serous, and synovial fluids: a textbook atlas. Chicago: American Society of Clinical Pathologists, 1986:117-27.

V. ANALYTICAL METHODS

A. Cell Parameters in Whole Blood (CBC) Coulter s880

1. Principle

The Coulter s880 System is a quantitative, automated hematology analyzer for in-vitro diagnostic use in clinical laboratories. It is intended for the quantitative determination of the following hematologic parameters: WBC, RBC, HGB, HCT, MCV, MCH, MCHC, and PLT.

A sample of anticoagulated venous whole blood is required to determine the blood parameters. The whole blood is automatically diluted by the instrument and prepared for analysis. White blood cells (leukocytes) and hemoglobin concentration are determined via the WBC dilution, and RBC and PLT are determined via the RBC dilution. The particle counting is accomplished by the impedance principle.

2. Specimen

a. Blood is collected in sodium or potassium EDTA.

b. Macro Tubes (3, 5, or 7 ml)--fill until vacuum is exhausted. Analyze within 4 hours of collection.

c. Micro Tubes--fill with 200-300 ul of whole blood. Analyze within 1 hour of collection.

d. Specimens may be stored at 2-6 ^{o}C for up to 24 hours for testing of all parameters except platelets. (Platelet counts must be stored at room temperature.)

3. Reagents

The following reagents are stable at room temperature until expiration date stated on container:

a. Isoton III, CMS Catalog no. 165-951 (Diluent)
b. Lyse III, CMS Catalog no. 172-940 (Lysing Agent)
c. Isoterge III, CMS Catalog no. 179-630 (Cleansing Agent)

4. Calibration

Initial calibration will be done on five normal samples, each run in triplicate. Calibration will be done on analysis of WBC, RBC, HGB, HCT, and platelets.

All five samples will be run four times on the analyzer. The first result will be discarded and the last three averaged.

Calibration will be checked daily by running three participant's specimens in triplicate for WBC, RBC, HGB, HCT, and platelets. Percent differences between the primary methods and the analyzer will be averaged and recorded.

Action will be taken when the analyzer results deviate by more than 3% for RBC, HGB, and HCT, 7% for WBC, and 10% for platelets.

5. Quality Control Material

   a. Normal (Catalog no. 122) and elevated (Catalog no. 123) levels of stabilized human red blood cells. (Equinox controls made by Hematronix)

      (1) Store upright at 2-10 °C before and after opening.

      (2) Unopened--stable until expiration date (150 days from manufacture date).

      (3) Opened--stable for 16 days. Discard after 16 days. (Stable 12 hours at room temperature.)

      (4) Warm vials to room temperature (18-30 °C) before handling. Mix by inversion until cells have been resuspended.

      (5) Place on mixer for 5 minutes, one time only when first opened.

      (6) Immediately before assaying, invert vial five times.

      (7) Run each level of control in quadruplicate each analytical run.

6. Procedure

   a. Preliminary Procedure:

      (1) Inspect the dilutor for disconnected or pinched tubing.

      (2) Turn on optic lamp.

      (3) Inspect the vacuum trap bottle on the pneumatic supply. (No liquid should be present.)

      (4) Turn on the pneumatic supply by pressing the "Power" button. Check the gauges:

         (a) Pressure--58-60
         (b) 5 PSI--5
         (c) 30 PSI--30
         (d) Vacuum--20

(5) Verify that the meniscus of the manometer is on the black reference mark.

(6) Press RES 1 and RES 2 simultaneously to reset the computer. (SELECT FUNCTION will appear on the digital readout if the reset was done properly.)

(7) Press DRAIN and verify that the aperture baths and vacuum isolator drain into the waste chamber.

(8) Press LYSE and verify that the lytic reagent enters the WBC bath without bubbles in the reagent line. Press DRAIN again.

(9) Press RINSE and verify that a) the apertures fill with diluent, b) that the waste chamber drains, and c) that the BSV rotates first to the right and then to the left.

(10) Press START UP. When the cycle is completed, press PRINT and log the results

(11) Set the date and test number (000).

(12) Prime the instrument with whole blood by sampling any specimen two times.

b. CBC Procedure:

(1) Mix samples at least 10 minutes on the mechanical blood mixer, but no more than 30 minutes. Mix controls no more than 5 minutes before initially opening vial. (Once vial is opened, do not place on mechanical mixer again.)

(2) Insert into the printer a report form with the participant identification written at the top.

(3) Invert each control or participant sample five times after removing them from mixer and immediately before the aspirating procedure.

(4) Place the specimen tube under the whole blood aspiration tube, inserting the aspiration tube 5-10 mm below the surface of the blood.

(5) Depress the WHOLE BLOOD button. Once the WIPE TIP appears on the digital display, remove the specimen.

(6) Wipe the aspiration tube with a damp Kimwipe.

(7) The next sample can be aspirated when the SELECT FUNCTION appears on the digital display.

(8) The results will be automatically printed. Each sample is run in duplicate. The first result is considered a prime and the second the accurate result. (Note: Both printouts must show logical agreement or the test must be repeated.)

7. References

a. Coulter Electronics. Coulter counter model s880, product reference manual. Hialeah, Florida: Coulter Electronics, 1985.

b. Coulter Electronics. Coulter course guide, Model s880. Hialeah, Florida: Coulter Electronics, 1985.

c. Gilmer PR, Williams LJ, Koepke JA, Bull BS. Calibration methods for automated hematology instruments. Am J Clin Pathol 1977; 68 (suppl 1):185-90.

d. Wintrobe MM. The approach to hematologic problems. In: Clinical hematology. 7th ed. Philadelphia: Lea & Febiger, 1974:3-38.

V. ANALYTICAL METHODS

B. Differential Counts of Whole Blood Smears
Geometric Data Hematrak
Automated Counter

1. Principle

The Hematrak incorporates a mathematical approach to morphologic analysis and a high resolution, three-color video scanning system that detects significant leukocyte morphologic features.

The video scanner searches the microscope's field until the color analyzer locates a nucleated cell. The digitized image of that cell is passed into the image memory. At this time, the morphological analyzer takes over, making significant measurements of each cell and performing morphologic analysis. Included in this analysis are the nucleus, nuclear:cytoplasm ratio, chromatic pattern, and cytoplasm.

The statistically derived and weighted factors are passed into the recognition computer where they are compared with preprogrammed criteria in the reference memory. The factors that make up the reference memory were developed by the analysis of hundreds of thousands of cells. By matching each cell's criteria against the reference memory, a classification is assigned to that cell by the instrument.

2. Specimen

a. Collect blood in sodium or potassium ethylenediamine tetra-acetate.

b. Store at room temperature until slide is made, no longer than 4 hours.

c. Make a spun smear, using the Hemaspinner. (See Hemaspinner procedure.)

d. Stain with Wright's stain, using the Hemastainer. (See Hemastainer procedure.)

e. Place finished slides in frames, five to a frame, frosted end at the top. Push all slides tightly to the left and up.

3. Reagents and Equipment

a. Hematrak immersion oil, Geometric Data, Catalog no. 20-107-06, Type HT. Store at room temperature.

b. Hematrak slide frames, Geometric Data, Catalog no. 20-108-02.

4. Calibration

No calibration is needed with this instrument. All parameters are preset and programmed by the manufacturer before the instrument is shipped.

5. Quality Control Material

Two slides have been prepared from two separate blood specimens. These will be run in quadruplicate each analytical run.

6. Procedure

a. Preliminary Procedure

(1) No start-up needed; instrument remains on at all times.

b. Differential Procedure

(1) Press DIFF on Hematrak CRT. LOOK AT THE SCREEN! It should say: "Operator ID, Date, WBC 200, RBC 200, spun slide." If it says "Re-cap review," insert slides. Press GO. Press DIFF again. If nothing changes, press RECELL, then DIFF.

(2) Frame slides, number frames consecutively, enter frame number and slide sample numbers on the log sheet.

(3) Place frame(s) in hopper.

(4) Press ID. On page 1 change operator number and date. Change WBC and RBC to 400. Enter the frame number on the second page and slide number. Enter sample number of slide 1, then second slide, etc. After fifth slide, enter frame 2. Slide 1 shows up automatically. If you have more than one frame, continue entering sample numbers until you have entered all of them. If you make a mistake, press DELETE before ENTER and correct your error. If you ENTER, then notice the mistake, press ID (light goes off), ID (light goes on), and go through by pressing ENTER until you come to the error. Press DELETE, correct number, enter.

(5) When all numbers are entered, press ID (light goes off).

(6) Now press GO. All frames will go through automatically. Allow 1 minute per slide. (You may enter the first frame, start it through then enter the ID numbers on the remaining frames.)

c. RECAP Review

(1) After all frames are done, the slides not meeting the preset criteria for "passing" will be flagged for review. Flagging

is indicated on the automatic printout and on the screen of the Hematrak CRT. A dot means no review, an F means review.

(2) Remove the frames from the bin, place in order in the hopper. Press GO. Enter first frame number (number, enter).

(3) The Hematrak automatically goes to the first slide in that frame that needs review and focuses on the first cell for review. Look through the microscope, adjust the focus as needed and identify cell (or just GO for smudges, stains, etc.) with the proper diff key. Press GO. The next cell is found.

Identify, GO. When done, the bell rings.

(4) Press COMPLETE. The next slide for review is automatically found. Continue as in step (3) until the frame is completed.

(5) The screen asks for the next frame number. Enter the number and continue as above through all the frames.

d. Morphological Review of Slides

(1) All slides must be reviewed for the presence of hypersegmentation, basophilic stippling, rouleaux, and Howell-Jolly bodies. Also check the printout from the Hematrak for any RBC morphology comments called by the instrument. Verify these and make a note on any RBC morphologic abnormalities you observe.

(2) Review slides for morphology during RECAP Review (section c under Procedure). Flag all slides not flagged by the Hematrak by entering an F for each one. Comments are written on the worksheet in the space to the right of patient information.

(3) Morphology information is entered into the computer manually. See the comment information sheets for type of answers used.

7. Comments to Use In Reporting

(Enter with actual abbreviation designated) OIF* - Oil Immersion Field.

Polychromasia*

SL (slight) = 2-4 cells/OIF*
MOD (moderate) = 5-6 cells/OIF
MKD (marked) = >6 cells/OIF

Hypochromasia*

SL = 10%-24%
MOD = 25%-49%
MKD = 50%+

Poikilocytosis*

SL = 4-10 cells/OIF
MOD = 11-20 cells/OIF
MKD = >20 cells/OIF

Rouleaux present are noted here in conjunction with the gradation of poikilocytosis.

Anisocytosis, Microcytosis, Macrocytosis*

SL = 5%-20%
MOD = 21%-40%
MKD = >40%

Basophilic Stippling

Rare = 1 or 2 cells/seen on scan
SL = 3 or 4 cells/seen on scan
MOD = 1 per OIF*
MKD = >1 per OIF*

Hypersegmentation

1+ = >5 polymorphonuclear neutrophils with five lobes/100 cells
2+ = 1 polymorphonuclear neutrophil with six or more lobes/100 cells
3+ = Combination of 1+ and 2+
4+ = Large numbers of 1+ and 2+

Howell-Jolly Bodies

Rare = 1 or 2 on scan
Few = 3 or 4 on scan
MOD = 1 per OIF*
MANY = >1 per OIF*

8. Reference

Geometric Data. Hematrak automated differential system, model 590 operator's manual. Wayne, Pennsylvania: Geometric Data, 1984.

V. ANALYTICAL METHODS

C. Prothrombin Time in Plasma
MLA 700 Automatic Coagulation Timer

1. Principle

The prothrombin time assesses extrinsic and common pathways in coagulation and is used to monitor antithrombin drugs.

MLA has developed the photometric clot detection system. Light from a calibrated, carefully controlled light source passes through a collimator, in which the light is focused into a parallel beam. The light illuminates the sample, and the light passing through falls on a photo detector, which converts the light intensity into an electrical signal.

As the plasma sample clots in response to the addition of thromboplastin, it becomes more opaque, and the light falling on the photo detector decreases. This alters the electrical signal. A sensitive amplifier processes the signal, and the instrument's microprocessor determines when a clot has started to form. The instrument then records the time elapsed between the addition of reagent and the start of clotting. This is the clot time.

2. Specimen

a. Collect blood in 3.8% sodium citrate, anticoagulated tubes. (Ratio 9:1, 4.5 ml whole blood to 0.5 ml citrate solution.)

b. Keep specimen cool. Spin as soon as possible. Separate the plasma into 10- x 75-mm plastic tube with cap.

c. Keep plasma cool (2-8 °C) until analyzed.

d. Analyze within 2 hours or freeze at -70 °C.

e. Minimum sample required: 0.2 ml plasma.

3. Reagents and Equipment

a. MLA 700, Model E-650, serial no. 205.

b. Plastic tubes with caps, 12 X 75 mm

c. Plastic cuvettes, red, Scientific Products catalog no. B 4163-7.

d. Reservoir and heat exchange tubing, Scientific Products catalog no. B 4163-2.

4. Quality Control and Other Material

a. Controls: Citrol Level I - Scientific Products catalog no. B4224-10, Dade
Citrol Level II - Scientific Products catalog no. B4224-20, Dade

(1) Lyophilized preparations of human plasma, stabilizers, and buffers.
(2) Store unopened vials at 2-8 °C.
(3) Remove from refrigerator and allow to warm to room temperature.
(4) Reconstitute with 1.0 ml distilled or deionized water. Stopper vial. Label with date and time and initial and allow to stand at room temperature until dissolved, approximately 15 minutes. Invert gently to mix. DO NOT SHAKE.
(5) Reconstituted material is stable 8 hours. Store at 2-8 °C when not in use.

b. Thromboplastin C: Scientific Products catalog no. B4216-50, Dade

(1) Lyophilized acetone-dehydrated rabbit brain thromboplastin; 1.16 X 10-2.0M Ca++, stabilizers and preservative
(2) Store at 2-8 °C.
(3) Reconstitute vials with 10 ml deionized water. Label with date, time, and initials.
(4) Stable for 3 days if stored at 2-8 °C. Mix well.

5. Procedure

a. Turn on machine and allow 20-30 minutes for temperature to stabilize.

b. Reconstitute controls and thromboplastin-C.

c. Check the following systems:

(1) Waste bin - empty
(2) Test time - normal
(3) Lever set for 300-second test
(4) Light source set on B
(5) Lever on AUTO

d. Fill cup with a MAXIMUM of 8 ml of thrombo-C. Add magnetic stirrer.

e. Prime tubing:

(1) Check to make sure plastic fitting is seated properly in each reagent cup.

(2) Prime reagent through tubing back into reagent cup until all bubbles are out of the line. Note: One bubble near colored tip of line is supposed to be there.

(3) Replace reagent cup lid.

(4) Check to see if tubing is seated properly in the grooves.

(5) Place nozzle into nozzle holder marked TEST.

f. Run temperature check and confidence test:

(1) Press PT 1 button
(2) Press START button twice in succession.
(3) Printout should read:

(4) Confidence = PT--19.9 $\pm$ 0.2
(5) Temperature = cold block--15$\pm$3 °C
(6) Reagent cups--15$\pm$3 °C
(7) Heat transfer pump block--37.5$\pm$0.2 °C
(8) Heat block--37.5$\pm$0.2 °C

g. Place the necessary number of cuvettes into the carousel to include controls where scheduled. Start with the first space to the right of the decoder space.

h. Add 0.1-ml amounts of control and participant samples to each cuvette position. (Each cuvette runs in duplicate.) Prewet the tip before pipetting. Carefully wipe the tip after sampling.

i. Press the START button once.

j. Two short beeps will indicate the run is complete.

k. The results will be printed out on tape.

l. Discard reagents. Wash cup with distilled water and dry

m. Flush tubing with deionized water. Remove tubing.

n. Turn off machine.

6. References

a. Dade. Thromboplastin-C package insert. Aguada, Puerto Rico: Dade, 1982.

b. Medical Laboratory Automation. Electra 700 automatic coagulation timer, operator's manual. Mt. Vernon, New York: Medical Laboratory Automation, 1984.

V. ANALYTICAL METHODS

D. Erythrocyte Sedimentation Rate in Whole Blood

1. Principle

The erythrocyte sedimentation rate (ESR) test measures the settling of erythrocytes in human plasma over a specified time period. The reported numerical value is derived from measuring, in millimeters, the distance from the bottom of the surface meniscus to the top of the erythrocyte sediment in a column of anticoagulated blood that has remained perpendicular in a special purpose pipet for 60 minutes.

The empirical phenomenon depends on an interrelationship of a number of important inherent variables, including plasma protein composition and concentration and erythrocyte configuration.

In this ESR method an attempt has been made to control or eliminate the variability of environmental and technical factors that influence the results.

The method is based on the Westergren method and calls for dilution of blood with autologous plasma to a standardized hematocrit. This change renders the method insensitive to hematocrit variations and provides a linear response throughout the range of clinical interest.

2. Specimen

a. EDTA (Na) anticoagulated whole blood

b. Minimum sample size is 5 ml; optimum size is 7 ml.

c. Blood left at room temperature (19-25 °C) must be set up within 2 hours. If blood is kept at 4 °C, it must be set up within 6 hours.

3. Reagents and Equipment

a. Anticoagulant: Ethylene diamine tetra-acetic acid (Na) is used at a concentration of 1.4 to 1.6 mg/ml of blood.

b. The Westergren Pipet (Scientific Products, catalog no. B4504-1 [Dade]):

(1) The pipet must have the following dimensions:
Overall length: 300.5±0.5 mm
External diameter: 5.5±0.5 mm
Tube bore: 2.70±0.20 mm
Uniformity of tube bore: ±0.05 mm

(2) The pipet must have the following markings:

(a) The tube is inscribed "Westergren."

(b) The tube has an inscribed graduated scale extending over the lower 200±0.35 mm of the pipet. The graduations are numbered from 0 at the top end of the scale to at least 180, no less than 20 mm from the lower end of the pipet, in steps of 10 or less.

Maximal tolerated error between two adjacent millimeter markings is 0.2 mm; tolerated error of the total graduated scale is 0.35%.

(c) The 1-mm graduation marks are fine, clearly marked, permanent lines of uniform thickness (0.2 mm). Every 10th graduation mark is numbered.

(d) The lengths of longer graduation marks are projected equally at each end beyond shorter markings. Specifically, the lengths are:

(i) At the 0 mark, 0.9-1.0 times the tube circumference

(ii) At each single millimeter mark, 0.125 (1/8) times the tube circumference

(iii) At each 5-mm mark, 0.16 (1/6) times the tube circumference

(iv) At each 10-mm mark, 0.25 (1/4) times the tube circumference

(v) The tubes, if disposable, must be supplied clean and dry. The manufacturer must ensure, and provide data on request, that the tubes do not contain any factor that may affect their use in the ESR test compared with WSP of the glass Westergren tubes described above.

c. Pipet Rack:

(1) Equipped with a leveling bubble device.
(2) Constructed so that no leakage of the blood can occur.

4. Quality Control Material

Four participant samples will be run in duplicate each analytic run.

5. Procedure

a. Blood Collection:

(1) Collect whole blood in a 7-ml EDTA tube.
(2) Mix immediately and THOROUGHLY by gentle inversion.

b. Hematocrit Adjustment:

(1) Obtain an initial hematocrit on the UNADJUSTED blood sample:

(a) Spin for 3 minutes at 10,000-11,000 rpm in the microhematocrit centrifuge. Read hematocrit on the accompanying reader.

(b) Centrifuge the two equal aliquots of 2 ml each at 800 rpm for 5 minutes to adequately divide cells and plasma.

(c) Remove plasma from or add plasma to one of the aliquots to obtain a sample with a hematocrit of 0.35. To calculate the volume required, use the following formula:

$$\frac{2 \times \text{hematocrit}}{0.35 \text{ required}} - 2 = \text{ml plasma}$$

(2) Determine the hematocrit of the ADJUSTED blood sample [use procedure as described in 5b(1)].

c. Blood Cell Suspension:

(1) Gently resuspend the blood cells thoroughly by placing on a mechanical rocker for at least 10 minutes. Blood should remain rocking until ready to aspirate.

(2) Using a mechanical suction device, aspirate a bubble-free sample into a clean and dry Westergren pipet, exactly filling to the "0" mark.

d. Handling of Westergren pipet:

(1) Set up singly. Pick four of the samples and set them up in duplicate.

(2) Place the filled pipet in a vertical position in the pipet rack. Area should be free of vibrations, air draft, and direct sunlight.

e. Reading of test:

(1) After 60 minutes ±30 seconds read the distance of millimeters from the bottom of the plasma meniscus (which should be at "0") to the top of the column of sedimented erythrocytes. Do not include buffy coat with the erythrocyte column.

(2) Record the numerical value.

6. References

a. Bull BS. Is a standard ESR possible? Lab Med 1975; 6(no. 11):31-5.

b. Koepke JA, Bull BS, Van Assendelft OW. Reference procedure for the human erythrocyte sedimentation rate (E.S.R.) test. NCCLS, 1983; 3(no. 7):251-64.

V. ANALYTICAL METHODS

E. Routine Urinalysis in a Random Urine

1. Principle

Properly executed routine urinalysis will indicate the presence of renal disease (protein, cells, and casts), urinary tract infection and diabetes mellitus (glucose). The routine urinalysis should be done on a fairly concentrated specimen, which is usually the first morning specimen, especially when examined for protein and microscopic content.

2. Specimen Collection and Storage

The urine sample must be collected in a clean, dry container covered with a lid, and examined as soon as possible. Bacterial contamination may occur, and leukocytes and casts disintegrate as the speciman ages. Specimens should be examined within 1 hour of collection. If this is not possible, the specimen must be refrigerated at 4 °C until it is examined. Minimum volume is 10 ml although 12 ml is optimum for the test.

3. Reagents

a. Ames Multistix:	Store at room temperature. Good until expiration date on the bottle. Ames, Inc., catalog no. 2820
b. Count-10 Trol II:	Urine Control, (15-ml lyopholyzed specimen). Store at 2-8 °C. Good until expiration date on the bottle (unopened). V-Tech, Inc. catalog no. 10-0751.
c. Clinitek calibration strips:	Store at room temperature. Good until expiration date on the box. Ames, Inc., catalog no. 5382.
d. Distilled water	
e. 4% Sodium chloride:	Store at room temperature. Stable for 6 months.

Procedure for preparing 4% NaCl:

1. Weigh 4 g of dried NaCl crystals.

2. Dissolve in distilled water in a 100-ml volumetric flask.

3. Q.S. to 100 ml.

4. Quality Control Material

a. Source:

V-Tech, Inc., Count-10-Trol Level II. Each vial contains lyophilized material derived from human urine containing measured amounts of chemicals, stabilized human erythrocytes, and simulated leukocytes.

b. Preparation:

(1) Bring reagents to room temperature.
(2) Slowly vent cap on reagent vial to avoid reagent loss.
(3) Reconstitute each control vial with 15 ml of distilled water. Stable 5 days after reconstitution.
(4) Cap and swirl control vial for 20 seconds. Allow 20 to 30 minutes for complete reconstitution.
(5) Remix reagent before each use.

c. Storage:

Store controls at 2-8 °C. Reconstituted controls remain stable for 5 days at 2-8 °C. Between use, all controls should be stored at 2-8 °C. Unopened controls are stable until the expiration date on the bottle.

5. Procedure

a. Perform calibration on the Clinitek 200 and the Atago-USR-20 refractometer.

b. Prepare controls.

c. Mix specimen well.

d. Transfer 10-12 ml of patient urine and control to the plastic centrifuge tubes labeled with specimen sample number.

e. Record color (straw, yellow, amber, or specific color) and appearance (clear, hazy, cloudy, flocculent).

f. Perform and record the specific gravity using the USR-20-refractometer. Record the result to three decimal places (e.g., 1.024).

g. Perform chemical examination with a dipstick and the Ames Clinitek 200.

h. Centrifuge urine tube for 5 minutes at 2,000 rpm.

i. Decant tube without blotting.

j. Perform microscopic examination using the phase X10 lens and the phase X40 lens.

(1) Resuspend the sediment.
(2) Examine the drop of sediment on slide with 20 x 20 coverslip, using the phase X10 lens first. Look for casts and epithelial cells.

Note: It is necessary to distinguish between cast types. Switch to the phase X40 lens and carefully scan 10-12 separate fields of the slide. Estimate average range of WBC, RBC, and renal tubular cells per high power field (hpf).

(3) Standard nomenclature:

(a) Cells (RBC and WBC): Range of numbers hpf.
(b) Casts: Range of number per low power field (lpf).
(c) Epithelial cells: Report percentage of lpf.

k. Perform the daily shut-down procedure on the Clinitek 200 and Atago Refractometer.

6. Codes and Comments To Use In Reporting

Assay Name:

Hemoglobin, Bilirubin

Codes	Comments
544	Negative
670	Small amount
406	Moderate amount
407	Large amount
2170	Trace

Ketones (mg/dl)

544	Negative
5369	5-15 (trace)
5370	15-40
5371	40-80
5372	>80

Urobilinogen (mg/dl)

Codes	Comments
5379	0.2-1 (- normal)
5380	1-2
5381	2-4
5374	4-8
5375	8-12
5382	>12

Protein (mg/dl)

544	Negative
2170	Trace
5373	30-100
5378	100-300
5363	>300

Glucose (mg/dl)

544	Negative
5376	100-250
5377	250-500
5367	500-1000
5368	>1000

Color

5326	Straw yellow (normal)
510	Amber
513	Red
5327	Blue-green
2156	Brown
5328	Dark yellow
5329	Yellow-orange
645	Orange
5330	Red-brown
2160	Yellow
511	Colorless

Appearance

145	Clear (normal)
517	Hazy
518	Cloudy
147	Turbid

RBC's and Leukocytes, Urine (cells/hpf)

Codes	Comments
396	None seen
1780	Rare
5331	Occasional
5332	Few = (1-5)
5333	1+ = (6-20)
5334	2+ = (21-50)
5335	3+ = (51-100)
5336	4+ = (>100)

Renal Tubular Cells (cells/lpf)

1780	Rare
5331	Occasional
5337	1+ = (1-5)
5338	2+ = (6-10)
5339	3+ = (11-30)
5340	4+ = (>30)

Casts, (Hyaline, Granular, WBC, RBC, Other)(no./lpf (X10))

1780	Rare
5331	Occasional
5337	1+ = (1-5)
5338	2+ = (6-10)
5339	3+ = (11-30)
5340	4+ = (>30)

Epithelial Cells, Urine (no./lpf)

1780	Rare
5331	Occasional
5341	Few = (<than 25% of field)
5342	1+ = (25% of field)
5343	2+ = (50% of field)
5344	3+ = (75% of field)
5345	4+ = (100% of field)
447	Packed

7. References

a. Ames Division, Miles Laboratories. Clinitek 200 operating manual. 4th ed. Elkhart, Indiana: Ames Division, Miles Laboratories, 1985.

b. Bradley M, Schumann GB. Examination of urine. IN: Henry JB, ed. Clinical diagnosis and management by laboratory methods. Philadelphia: WB Saunders, 1984:380-58.

c. NSG Precision. Instruction manual for the USR-20 refractometer. Hicksville, New York: NSG Precision, 1984.

d. V-Tech. Count-10-Trol urine control package insert. Pomona, California: V-Tech, 1984.

V. ANALYTICAL METHODS

F. D-Glucaric Acid in Urine
Ion-Exchange Chromatography

1. Principle

For the quantitative determination of D-glucaric acid, buffered urine specimens are added to an ion-exchange resin-filled column to separate D-glucaric acid from other interfering substances. After elution from the column, D-glucaric acid is oxidized with periodic acid to yield glyoxylic acid. Glyoxylic acid is then oxidatively coupled with phenylhydrazine to yield the dye, 1,5-diphenylformazan. Absorbance at 520 mm is read and concentration determined by comparison to standard curve absorbance.

D-glucaric acid is a product of glucuronic acid metabolism in the liver. Urinary excretion reflects the activity of hepatic enzymes that hydroxylate and detoxify many foreign substances.

Elevated levels are observed after the administration of drugs known to induce enzyme activity, and anticonvulsant drugs and in association with exposure to insecticides.

L-ascorbic acid (vitamin C) is know to falsely elevate D-glucaric acid levels obtained in this method; therefore all urines are prescreened for ascorbic acid by using a dipstick method.

2. Specimen Collection and Storage

a. No participant preparation is necessary.

b. A well-mixed aliquot (4-10 ml) of a 12-hour. urine collection is used for this test. Urine specimens are collected in containers with 2 of sodium carbonate. The specimen should be protected from light and kept cold during collection.

c. The specimen is stable for 24 hours at 2-8 °C. For longer storage, freeze at -20 °C.

3. Reagents, Supplies and Equipment

a. Glass test tubes, disposable: 16 X 100 mm, 16 X 150 mm, 10 X 75 mm

b. Pipets:
Eppendorf pipet, 12.5-ml tips
MLA, 1 ml with disposable tips
Accuprep (Beckman) capable of dispensing 1-1,000 ul
Repipet, 5-ml (for addition of eluant to column)

c. Class A volumetric flasks: 2L, 1L, 100 ml, 50 ml, 10 ml

d. Analytical balance (Mettler AE 166)

e. Spectrophotometer having band width of 1-2 nm, 1.0-cm light path (Beckman DU-7)

f. Millipore filters

g. Draining rack with waste container

h. Plastic transfer pipets

i. Magnetic stirrer

j. Plastic caps, 16 mm

k. Stix (ascorbic acid reagent strips) Ames

l. Funnels (BioRad)

m. Vortex mixer

n. Test-tube racks

o. Parafilm

p. Timer

q. Resin columns, AG 1X-8 resin, obtained from BioRad. Store at room temperature. Refer to expiration date on box.

r. Sodium borate 0.1M

(1) Dissolve in volumetric flasks:
(a) Sodium borate--305.96 g
Certified ACS, Fisher.
(b) Reagent water type I--8 L
(2) Place in polyethylene carboy. Label as "Sodium Borate 0.1M" with preparation date, technician ID, and an expiration date of 1 year. Store at room temperature.

s. Sodium borate 0.05 M/sodium sulfate 0.02 M

(1) Dissolve in volumetric flasks:
(a) Sodium borate--190.685 g
(b) Sodium sulfate--28.4080 g
Anhydrous, E M Science.
(c) Reagent water type I--10 L

(2) Place in polyethylene carboy, label as "Sodium Borate 0.05M/Sodium sulfate 0.02M" with preparation date, technician identification, and an expiration date of 1 year. Store at room temperature. This quantity is sufficient for 1 week.

t. Sodium borate 0.05M/sodium sulfate 0.1 M

(1) Dissolve in volumetric flasks:
(a) Sodium borate--190.685 g
(b) Sodium sulfate--142.040 g
(c) Reagent water type I--10 L

(2) Place in polyethylene carboy, label as "Sodium Borate 0.05 M sodium sulfate 0.1M" with preperation date, technician's initials, and an expiration date of 1 year. Store at room temperature. Aliquots are transferred to a repipet capable of dispensing 5 ml. Label as above.

u. Saccharic acid (potassium hydrogen d-glucarate), Sigma, store in desiccator at room temperature.

v. Potassium biphosphate 1.0 Molar

(1) Dissolve in a 100-ml volumetric flask:
(a) Potassium phosphate monobasic--13.609 g
(b) Technical grade, MCB.
(c) Reagent water type I--QS to 100 ml

(2) Transfer to glass bottle, label as "Potassium Biphosphate 1.0M" with preparation date, technician's initials, and an expiration date of 2 weeks.
Store at 2-8 °C. Mix well before using.

w. Periodic acid 0.01 Molar

(1) Dissolve in a 50-ml volumetric flask:
(a) Periodic acid--0.114 g
Sigma
(b) Reagent water type I-- QS to 50 ml

(2) Transfer to brown glass bottle, label as "Periodic Acid 0.01M" with preparation date, technician's initials, and an expiration date of 1 week. Store at 2-8 °C.

x. Phenylhydrazine Hydrochloride 1%

(1) Dissolve in a 100-ml volumetric flask:
(a) Phenylhydrazine HCl--1.0 g
MCB
(b) Reagent water Type I--QS to 100 ml.

(2) Transfer to brown glass bottle, label as "Phenylhydrazine HCl 1%" with preparation date, technician's initials, and an expiration date of 1 week. Store at 2-8 °C.

y. Hydrochloric Acid, conc. Reagent Grade, MCB Reagents. Store under hood.

z. Potassium ferricyanide 5%

(1) Dissolve in a 100-ml volumetric flask:
(a) Potassium ferricyanide--5.0 g
Technical grade, MCB.
(b) Reagent water type I-QS to 100 ml

(2) Transfer to a brown glass bottle, label as "Potassium Ferricyanide 5%" with preparation date, technician's initials, an expiration date of 1 week. Store at 2-8 °C.

aa. Ascorbic Acid Control: 25 mg/dl

(1) Dissolve in an 1L volumetric flask:
(a) 250 mg ascorbic acid A.C.S. MCB reagents
(b) Reagent water type I-QS to 1 L

(2) Aliquot approximately 3 ml in 10- x 75-mm tubes and store at -20 °C. Stable for 6 months.

bb. Ascorbic acid dipsticks (Uralyte GK + A) Medical Sales Associates, catalog no. 8001.

4. Calibration

a. D-dlucaric acid stock std. 1,000 ug/ml

(1) Dissolve in a 100-ml volumetric flask:
(a) D-saccharic acid--100 mg
(b) Reagent water type I--QS to 100 ml

(2) Aliquot 1-2 ml in 10 X 75 mm tubes and store at -20 °C. Stable for 3 months.

b. Using the Beckman Accuprep, dilute working standards daily as follows:

Prepare the working standards during the final elution (step 6d(3) of the procedure that follows).

c. Prepare 100 ug/ml working stock standard:

Dilute 1 ml (use a 1 ml volumetric pipette) of the 1,000 ug/ml stock in a 10-ml volumetric flask, QS to 10 ml with water reagent type I.

Prepare Standards:

D-glucarate std ug/ml	ml of 100 ug/ml Stock Standard	ml of 0.05 m NaBorate/ 0.1 m NaSulfate
2	0.200	9.8
4	0.400	9.6
6	0.600	9.4
8	0.800	9.2
10	1.000	9.0

Standards at the 2-, 4-, 6-, 8-, and 10-ug/ml levels are run in duplicate with each assay.

5. Quality Control Material

A control urine will be run in duplicate at the beginning and end of each analytical run. The control urine used is Lyphochek Quantitative Urine Control Abnormal Level II by BioRad. The control is reconstituted by using a 25-ml volumetric pipet and reagent water, type I. It is made up at the beginning of each week and then aliquoted and frozen for each day's use. A positive and negative ascorbic acid control will be run at the beginning and end of the ascorbic acid screening procedure and ascorbic acid results for controls and participant samples will be recorded on the worklist.

6. Procedure

a. Allow all reagents, standards, and participant sample specimens to come to room temperature before testing.

b. Label 1 BioRad column for each participant and control. Shake column vigorously until resin is completely suspended. No resin should remain in tip when the column is inverted. Snap off ends, attach funnels, place in rack, and drain into a waste container. Do not insert pipets or other objects into the columns to further mix the contents while they are draining.

c. Screening for Ascorbic Acid:

(1) In addition to each participant sample, a positive ascorbic acid control and a negative control (reagent water type I) must be tested for ascorbic acid. Run these controls at the beginning and end of each analytic run.
(2) Completely immerse the reagent area of an ascorbic acid dipstick in the well-mixed, uncentrifuged urine or control sample.
(3) Draw the edge of the strip against the side of the container to remove any excess liquid.

(4) Compare the color of the reagent area with the color chart on the bottle label at exactly 60 seconds. Hold the strip close to the color blocks and match carefully.
(5) Record results in the 0-10 mg/dl range as negative. Any results of 25 mg/dl or greater are to be recorded on the D-glucaric worksheet as positive and the D-glucaric result NOT reported. Any positive ascorbic acid screen is to be reported to the QC supervisor.

d. Separation of D-glucaric Acid:

(1) Label one 16- x 100-mm test tube for each participant and control. Use a 1-ml MLA to place 2 ml of the well-mixed 12-hour urine or control into its tube. Using an Eppendorf pipet, add 2 ml of 0.1M sodium borate to each tube. Vortex and let stand at room temperature 15 minutes.
(2) Pour the urine mixture onto each drained column. Allow the samples to be completely absorbed on the resin (about 20 minutes) before proceeding.
(3) Wash the columns with 100 ml of 0.05 M sodium borate-0.02 M sodium sulfate. Drain completely into the waste receptacle (about 3 hours).
(4) Label one 16- X 150-mm test tube for each participant/control and place under the columns. Elute D-glucaric acid with 15 ml (use the 5-ml repipet) 0.05M sodium borate/0.1 sodium sulfate. Let drain completely into tubes (about 1 hour).

e. Colorimetric Procedure:

Make up daily standards as described in the calibration section.

(1) Cover with Parafilm and mix the eluate well by inversion. With an MLA, place 1 ml in each of two labeled 16- X 100-mm test tubes.
(2) With an MLA, place 1 ml of each of the standards into duplicate labeled 16- X 100-mm test tubes. Two zero standards (reagent blank) are prepared by pipetting 1 ml of the 0.05M sodium borate/0.1M sodium sulfate buffer into two labeled tubes.
(3) Add 200 ul (use Accuprep program 1) of 1.0M potassium biphosphate to each tube and vortex. Make sure the potassium biphosphate reagent is well-mixed before using.
(4) Add 20 ul (use Accuprep program 4) of 0.01M periodic acid, vortex again and let stand at room temperature. for 15 minutes.
(5) Add 200 ul (use Accuprep program 1) of 1% phenylhydrazine hydrochloride, vortex, and let stand at room temperature. for 10 minutes.
(6) Using the accuprep program 2, add 1 ml of concentrated HCl; vortex, and follow immediately with the addition of 200 ul 5% potassium ferricyanide (Accuprep). Vortex and let stand at room temperature 5-10 minutes.
(7) Measure and record the absorbancy at 520 nm against the reagent blank.

7. Calibrations

a. Use the linear regression program in the CHC or the IBM PC/XT computer to evaluate the standard curve and obtain participant results (in ug/ml).

b. After this result and the urine creatinine (creat) results have been verified, the force calculations mode is used to perform the following calculation:

$$\text{D-Glucaric Acid (in mg/g creat)} = \frac{\text{Uncorrected (D-Glucaric Acid (in ug/ml)} \times 7.5)}{\text{Urine Creat/100 (in mg/dl)}}$$

c. Each duplicate control result is multiplied by 7.5 to correct for the dilution factor.

8. References

a. Hunter J, Maxwell JD, Carella M, Stewart DA. Urinary D-glucaric acid excretion as a test for hepatic enzyme induction in man. Lancet 1971;1:572-5.

b. Ishidate M, Matsui M, Okada M. Biochemical studies on glucuronic acid and glucaric acid: quantitative chemical determination of D-glucaric acid in urine. Anal Biochem 1965; 11:176-89.

c. March J, Turner WJ, Shanley J, Field J. Values for urinary excretion of D-glucaric acid by normal individuals. Clin Chem 1974; 20(No.9):1155-8.

d. McFadden BA, Howes WV. The determination of glyoxylic acid in biological systems. Anal Biochem 1960; 1:240-8.