Manual 10

Clinical Chemistry Determinations

The National Heart, Lung, and Blood Institute
of the National Institutes of Health

ARIC PROTOCOL

Manual 10

Clinical Chemistry Determinations

Visit 2

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FOREWORD

This manual entitled, Clinical Chemistry Determinations, is one of a series of protocols and manuals of operation for the Atherosclerosis Risk in Communities (ARIC) Study. The complexity of the ARIC Study requires that a sizeable number of procedures be described, thus this rather extensive set of materials has been organized into the set of manuals listed below. Manual 1 provides the background, organization, and general objectives of the ARIC Study. Manuals 2 and 3 describe the operation of the Cohort and Surveillance Components of the study. Detailed Manuals of Operation for specific procedures, including reading centers and central laboratories, make up Manuals 4 through 11. Manual 12 on Quality Assurance and Quality Control contains a general description of the study's approach to quality assurance as well as specific protocols for each of the study procedures.

The version status of each manual is printed on the title sheet. The first edition of each manual is Version 1.0. Subsequent modifications of Version 1 (pages updated, pages added, or pages deleted) are indicated as Versions 1.1, 1.2, and so on, and are described in detail in the Revision Log located immediately after the title page. When revisions are substantial enough to require a new printing of the manual, the version number will be updated (e.g., Version 2.0) on the title page.

ARIC Study Protocols and Manuals of Operation

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6	Ultrasound Assessment a. Ultrasound Scanning b. Ultrasound B-mode Image Reading Protocol
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Manual 10. Clinical Chemistry Determinations

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PREFACE

The Atherosclerosis Risk in Communities (ARIC) Study is a collaborative contract research program funded in 1986 by the National Heart, Lung, and Blood Institute. The Central Chemistry Laboratory at the University of Minnesota has contracted to provide the following analyses: glucose, creatinine, magnesium, hemoglobin Alc, uric acid, sodium, potassium, insulin. Insulin and "Alc" analyses are done for case-control only. Frozen serum collected at the field centers is shipped to the University of Minnesota Hospital Laboratories. The analytical methods and quality control programs (both internal and external) follow those of the University of Minnesota Hospital Laboratories. In addition, blind replicate samples are submitted from field centers as an additional means of establishing the validity and precision of the assays. This Manual outlines the instrumentation, analytical methodologies, calibration procedures, and quality control methods.

1. SPECIMEN HANDLING AND PROCESSING

1.1 Receipt of Specimens

1.1.1 Source

Specimens are sent from four field centers:

Field Center	Code	Operated by
Washington County, MD	W	Johns Hopkins University
Jackson, MS	J	University of Mississippi
Forsyth County, NC	F	University of North Carolina
Minneapolis Suburbs, MN	M	University of Minnesota

The University of Minnesota Hospital and Clinic Chemistry Laboratory is one of seven central agencies in the ARIC study. It participates as the Central Chemistry Laboratory for the study (CCL in this manual). The agency code for the Central Chemistry Laboratory is C.

1.1.2 Shipment and Arrival of Specimens

Samples are packed in dry ice and shipped in insulated styrofoam boxes. The boxes are shipped on Monday or Tuesday, regardless of the size of shipment. The number of donor specimens per shipment will vary from a very few specimens to forty or fifty. The CCL address that specimens are shipped to is:

ARIC Study - M. Fowler
ATTN: Room L275 Mayo (612-626-3938)
U of MN Hospital and Clinic
420 Delaware Street S.E.
Minneapolis, MN 55455

The samples received in a single shipment from a field center constitute a batch. A batch consists of all samples collected during a specific time interval as defined in ARIC protocol. Each batch is identified by a batch ID number.

Outstate field centers send specimens via an overnight courier. The courier delivers the specimen containers to the laboratory. The local field center at the University of Minnesota sends specimens by a local courier.

1.2 Unpacking the Styrofoam Box and Checking Contents

1.2.1 Contents. Each shipment (batch) contains the following:

1. Dry ice: CAUTION

2. Labeled 3"x6" storage bag containing five (5) 2ml white cap microtubes and one (1) red cap microtube. One bag for each donor.

3. Forms
   1. ARIC Shipping Form (Inventory File).
   2. ARIC Shipping Form - Face Sheet (Manual of Laboratory Operations I, Blood Drawing and Processing, Second Draft, page 50, 53).

1.2.2 Unpacking and Checking Contents

Supplies needed:

1. Basins to hold dry ice and test tube racks. The number needed will vary with batch size.

2. Four test tube racks with spaces numbered 1-10, 11-20, 21-30, 31-40 respectively, and with spaces for four tubes behind each number and 1 chilled rack labeled for Alc specimen.

3. Test tube blocks, prelabeled and chilled, red, K. Scientific, 50 spaces. One for each batch.

4. Storage boxes, from Kelvinator, Cat #33-0472 P. Storage box inserts, Cat #33-0476 P. These are for permanent storage at -70°C. The boxes are numbered sequentially and there are duplicate sets, i.e., number 001A, 001B, 001C, 001D, and 001A1c; 002A, 002B, 002C, 002D, and 002A1c. The "A" set is permanent storage for the ARIC study. The duplicate series are for additional tests on cases and controls.

   Spaces in a box are numbered sequentially starting at a front left corner, indicated by a red dot. A red dot is also put on the cover to match the corner of box and cover. A chart of the numbering sequence is taped on the cover to facilitate determining the location of the specimen tubes and the record keeping. The sides of the box are manually labeled with the numbers of the end of each row. The numbering sequence is as follows:

Row	Number	Row	Number
1	1-11	7	67-77
2	12-22	8	78-88
3	23-33	9	89-99
4	34-44	10	100-110
5	45-55	11	111-121
6	56-66		

5. Clip Board with the current Chemistry Batch Log form and the Specimen Inventory by Storage Box form.

NOTE: Specimens must not be allowed to thaw!

Procedure:

1. Gather and prepare the items needed for unpacking.

2. Open container and remove paper forms. Put cover back on. These forms and/or diskette/PC generated forms are used for recording arrival state of a batch and for checking the contents of the shipment.

3. The ARIC Shipping Form is used to check off specimens as they are removed from the styrofoam container. Before removing the specimens, do the following:

   a. Number the participants sequentially, starting with number one, in the left margin of the Shipping Form.
   b. Record the storage box and position number for each specimen on the Shipping Form.
   c. Record batch number and date received on red block.
   d. Record on the top of the Shipping Form the batch number and the date received.

4. Note the condition of the total shipment as the box is opened. Fill in the appropriate part of the ARIC Shipping Log Face Sheet.

5. Because specimens cannot be allowed to thaw, leave some of the dry ice in the container. Put part of the dry ice in the basins. If more dry ice is needed, it can be borrowed from UMHC Central Specimen Receiving and Processing (CSRP). Place test tube racks, batch rack and Alc rack on the dry ice in the basins.

   Remove bags one at a time.

   a. Check the identification on the outside of the bag against the identification on the six tubes in the bag.
   b. Note any discrepancies.
   c. Note the transport condition of each donor specimen. This information is part of each donor's ARIC record. Using the list below as a guide, make a notation on the Shipping Form if the specimen is other than (00) arrived frozen and unbroken. This information is entered into the computer later.
      00 - arrived frozen and unbroken
      01 - arrived frozen, but vial broken
      02 - thawed in transit, but vial unbroken
      03 - thawed in transit, vial broken
   d. Check the identification number on the tubes against the Shipping Form. Note the position number (see 4a above) of that donor on the list and put the four tubes in that numbered position in the appropriate test tube rack, one tube behind the other and the tubes to be run on Dacos or for Alc in appropriate slot in batch's red racks.

6. When the styrofoam box has been emptied, count the number of donor specimens received in the batch and check that the number agrees with the listing on the Shipping Form and Shipping Form Face Sheet. See section 1.2.4 for handling discrepancies.

7. Immediately put the red block containing the set of specimens to be used for analysis into the -70°C freezer.

8. At the time the red block is put into the -70°C freezer, remove the five (A-D, A1C) appropriate storage boxes from the -70°C freezer.

   a. Put the tubes in the same numbered position in the five boxes. The chart taped on the cover, and the pre-numbered sides will facilitate this.
   b. Put the first tube in the "A" box, the second tube in the "B" box, and continue with "C" and "D" boxes in the same manner.
   c. Return the storage boxes to the -70°C freezer.

9. After the specimens have been checked and are in -70°C storage, check the Chemistry Batch Log and the Specimen Inventory by Storage Box form for accuracy.

10. Copies are made of the Shipping Form and Shipping Form Face Sheet and are sent to the field center. Two additional copies are made of the Shipping Form for use in Central Chemistry Laboratory. One each will be used as follows:

    - As a work sheet to accompany specimens to the laboratory
    - To log storage of specimens when chemistry testing is complete.

1.2.3 Processing of Face/Log Sheet and Inventory Sheet

The original Shipping Form and Shipping Form Face Sheet for all batches received and any problem log forms are to be saved to provide an audit trail of specimens and data received. Put the forms received in one week in a manila folder dated for that week. When the work is completed, the folder is put into the ARIC file.

1.2.4 Reporting and Resolving Discrepancies

If any discrepancy is detected, the person preparing the batch is contacted by phone at the field center to resolve the problem. Document the problem on the Shipping Form.

1.3 Secure Safe Storage of Specimen

1. Immediately after checking the condition of the shipment and specimens, the specimens are put in the -70°C freezer in the ARIC Specimen Receiving and Processing area. The tubes to be analyzed are stored in ARIC batches in red blocks. Tubes for back-up and long-term storage are kept in separate storage boxes.

1.4 Preparation of Specimens for Analysis

Remove appropriate specimens from ARIC freezer and deliver to the DACOS Laboratory. Allow to thaw at room temperature. Mix well by inversion.

1.5 Specimen Distribution and Workflow

1. A copy of the Shipping Form with ARIC identification numbers will accompany specimens to the work place to help identify batches and to serve as a work list.

2. Specimens are run on the DACOS (Sec. 2.5.3, #2). A result report form is printed out by the DACOS for each participant.

3. The result printouts are reviewed for alert values, which are recorded on an Alert Value form. Also recorded with the alert values are any abnormal values. This Alert Value form is eventually taken to the PC and the results transmitted by electronic mail to the field centers.

4. Manually transcribed on the DACOS printout is each participant's specimen appearance code, transport condition code, and location in a storage box.

5. The DACOS printout is taken to the PC. The appropriate Shipping Forms are also taken to the PC for the "date sample received" information.

1.6 Enter Results and Codes into the Hewlett Packard Vectra

Detailed instructions are in the Batch Data Entry System manual. Start by responding to the prompts on the screen. Enter results and codes for each participant. If results exceed limits programmed into the computer, a message will appear in the upper left corner. When a message appears, check the value entered against the Alert Value/Computerized Editing Limits column on page 66. If the value exceeds the limits of a test with asterisk, check the Alert Value form (see 2.5.4, #5) to make certain the alert value was recorded and the appropriate field center notified. Complete entering all results. Print a copy of each participant's results. These printed copies are checked against the DACOS printouts for entry errors. Run the exit program, which utilizes the back-up disk. When the batches received in a one-week period are entered and checked, mail the work disc to the Coordinating Center. Discs are mailed on Thursdays.

1.7 Reporting Notification/Alert Values

Report all results that exceed the notification limits (see Tables 1 and 2) to the appropriate field center by electronic mail. To obtain acknowledgement the Request Receipt option of the Address/Store menu is used. This provides a method of flagging messages for automatic return receipts.

1.8 Reporting Results to the Field Center

One copy is made of each participant's computer printout. These copies are mailed to the appropriate field center.

1.9 Long-Term Storage of Remaining Tube(s)

Tubes will be stored in the ARIC -70°C freezer in L271 Mayo.

TABLE 1. Central Chemistry Laboratory Results

Analyte	Report Format	Units	Alert Value and Computerized Editing Limits[1]	Reference Ranges[2]	Sex
Sodium	XXX.	mmol/L	130 - 155*	136 - 147	M/F
Potassium	XX.X	mmol/L	3.0 - 6.0*	3.5 - 5.2	M/F
Glucose[3]	XXXX.	mg/dL	60 - 200*	70 - 130	M/F
Creatinine	XX.X	mg/dL	> 2.0*	< 1.5 < 1.3	Male Female
Magnesium	XX.X	meg/L	> 3.0*	1.3 - 1.9	M/F
Uric Acid	XX.X	mg/dL	(no alert values)	< 9.2 < 8.1	Male Female
Insulin	XXX.	mU/L	0 - 200	< 25	M/F
Hemoglobin Alc	XX.X	%	(no alert values)	4.3 - 6.1	M/F

[1]This column lists the Editing Limits used for the Computerized Data Entry System for all of the chemistry tests. Six of the eight chemistry procedures have alert value limits and the alert value is the computerized editing limit. An asterisk denotes those analytes with alert value limits.

[2]The reference ranges were established for each analyte by taking the lowest and highest value from either the literature or locally established values of Table 2.

[3]In June 1987, 300 ARIC participant glucose results were plotted as a frequency distribution. The upper limit for the reference range was arbitrarily selected as 130 mgd/L (87.0th percentile). The lower limit was left as the lower limit of published reference ranges.

*Values outside of these limits will show at the top of the computer screen. Alert values for tests with an asterisk will be sent by electronic mail to the field centers.

TABLE 2. Comparative Adult Reference Ranges

Analyte	Sex	Literature[1]	University of Minnesota Hospital and Clinic	
			Range	Source
Sodium	M/F	136 - 146 mmol/L	138 - 147 mmol/L	2
Potassium	M/F	3.5 - 5.1 mmol/L	3.7 - 5.2 mmol/L	2
Glucose	M/F	70 - 105 mg/dL	72 - 106 mg/dL	2
Creatinine	M	0.6 - 1.2 mg/dL	0.5 - 1.3 mg/dL	2
	F	0.5 - 1.1 mg/dL	0.5 - 1.0 mg/dL	
Magnesium	M/F	1.3 - 2.1 mEq/L	1.4 - 1.9 mEq/L	2
Uric Acid	M	3.5 - 7.2 mg/dL	4.3 - 7.6 mg/dL	3
	F	2.6 - 6.0 mg/dL	3.2 - 6.0 mg/dL	
Insulin	M/F	6 - 24 µIU/mL 12-hour fast	0 - 20 mU/L 12-hour fast	4
Hemoglobin Alc	M/F	3.0 - 6.0 %	4.3 - 6.1 %	5

References

1. Tietz NW. Textbook of Clinical Chemistry. Philadelphia: W.B. Saunders Company, 1810, 1986.

2. Based on ± 2 SD from the mean of results on specimens collected in 1972 and 1973 from normal ambulatory volunteers living in a southeastern Minnesota community. Ranges were put in use in approximately 1973.

3. Based on ± 1.282 SD (90% confidence levels) from the mean of results on specimens from blood donors at UMHC. Established March 1967.

4. Based on ± 2 SD from the mean of results on specimens from students and employees of UMHC. All specimens were 8 am fasting specimens. Established October 1983.

5. Based on 95% confidence levels established by BioRad Clinical Division, Hercules, CA using the described Diamat HPLC methodology.

2. DACOS

2.1 Introduction

The Coulter Discrete Analyzer with Continuous Optical Scanning (DACOS) is a discrete, multi-test, sequential, programmable analyzer that performs panels, selective tests, continuous or batch testing, and STATS in any sequence you select. The DACOS measures cuvette blanks, serum blanks, reagent blanks, and continually monitors each chemical analysis. Its optical system rotates eight different optical paths past 120 indexing acrylic cuvettes. The system operates at the rate of 450 tests per hour regardless of testing methodology. The DACOS Analyzer plus LYTES Option is an analyzer designed for the measurement of sodium and potassium.

2.2 Specimen

2.2.1 Requirement

Serum is used for all procedures. Study participants will have taken nothing by mouth other than water for at least 12 hours prior to having blood drawn. Other specific information about the specimen handling, etc., prior to its arrival here is found in the Blood Drawing and Processing Section of the Manual of Laboratory Operations.

2.2.2 Checking Appearance of Specimen

The technologist running the DACOS procedure monitors the appearance of every specimen and enters the appropriate appearance code into the computer. The codes are as follows:

10 = color normal
11 = hemolyzed
12 = icteric
13 = slightly lipemic, specimen not ultracentrifuged
14 = hemolyzed and lipemic
15 = icteric and lipemic
16 = lipemic, specimen ultracentrifuged

2.2.3 Interferences

1. HEMOGLOBIN

Potassium - falsely elevated at a hemoglobin level ≥ 150 mg/dL. This is considered moderately hemolyzed and will elevate the K+ by 0.24 mmol/L at a level of 4.8 mmol/L. (Hemolysis can be detected by the naked eye at 25 mg/dL.)

Magnesium - Although the magnesium concentration in erythrocytes is about three times that in serum, our studies on hemolyzed specimens by AAS have shown no significant increases in magnesium levels.

Calcium - The Coulter manual states that specimens should be free from hemolysis. However, it also states that no significant hemolysis interference is seen up to a level equivalent to 500 mg/dL of hemoglobin (red cell lysate).

2. LIPEMIA

All of the procedures are affected by gross lipemia. Creatinine is affected by moderate lipemia with a resulting decrease in results of about 0.3 mg/dL. All lipemic specimens with more than moderate lipemia (unable to read printed page through a 12 mm tube) are ultracentrifuged in a Beckman airfuge. When appearance code 16 is used, specimens are ultracentrifuged prior to analysis for all analytes.

3. BILIRUBIN

Creatinine - Direct bilirubin, 10 mg/dL diglucuronide, can cause a decrease in results of 0.3 mg/dL. Total bilirubin, 20 mg/dL crystalline, can cause a decrease in results of 0.3 mg/dL.

2.3 Equipment and Supplies

1. DACOS Analyzer with Lytes Module, Coulter Electronics, Inc., Hialeah, FL 33012

2. DACOS sample trays, 40/pkg, Curtin Matheson Scientific Inc. (CMS), #156-463, Coulter Diagnostic Division (CDD) #7546717

3. Cuvettes, 150/pkg, CDD #7546662

4. Pointer Ribbon Cartridge, CMS #158303, CDD #7546742

5. Photometer Lamp Replacement Kit, CDD #6602560-8

6. Syringe - sample, CDD #25172035

7. Syringes - Reagent 1 and 2/Diluent, CDD #2517206-0

8. Syringe O-Rings - Reagent 1 and 2/Diluent, CDD #25231520

9. Syringe Plunger tips - Reagent 1 and 2/Diluent, CDD #2523034-5

10. Molykoti 33 grease, Dow Corning CDD #1604007

(See Table 3.1, DACOS Manual Vol. 1, for additional operator-replaceable parts.) For Electrolytes: plus LYTES Option

11. Potassium electrode, CDD #2906349-4

12. Reference electrode, CDD #2906352-4

13. Reference membrane, CDD #2906353-2

14. Sodium electrode, CDD #2966351-6

(See Table 5.1, DACOS plus LYTES Option Manual for additional operator-replaceable parts.)

2.4 Reagents

1. Water (NCCLS Type II)
   Service Deionization System (SD). The SDI process consists of a 5 micron prefilter followed by one activated carbon and two ion exchange tanks in series. This meets NCCLS Type II water requirements.

2. Cuvette Wash, 4 x 1500 ml, CMS #135-137, CDD #7546768.

3. Diluent; A=10 x 54 g and B=10 x 25 ml, CMS #242-891, CDD #7646876. Make fresh daily. To make: Add contents of Diluent vials A and B to 1500 mls of NCCLS Type II water. Mix well, then dilute to a volume of 1800 mls.

plus LYTES Option:

4. 2M KCL Reference Solution, 2 x 125 ml, CMS #237-529, CDD #7546828.

5. Rinse solution, 2 x 125 ml CMS #237-537, CDD #7546829.

6. Test Dye, 1 x 50 ml, CMS #237-560, CDD #7546831. To make: combine equal parts of test dye and Std. A. Make weekly.

7. 3% Bleach. Dilute 3 parts household bleach and 2 parts NCCLS Type 2. Note: Use Hilex bleach and not bleach obtained from Chemical Storehouse.

8. Reagents and calibrators for each method are listed on each procedure.

2.5 Instrument Operation

Operating instructions, DACOS Manual, Vol. II, Section 6 should be read before operating the instrument.

2.5.1 Pre-operational checks - instrument should be in WARM state.

1. Check that there are 120 good cuvettes.

2. Check wash solution dilution chamber and container.

3. Check diluent container. (Make fresh diluent every day.)

4. Check reagent storage carousel water level and temperature. Add tap water, if necessary, and fill to liquid fill ridge. Temperature should be 15°C ± 2°. CAUTION: DO NOT take temperature if the analyzer is priming or about to go into a prime (e.g., WARM ——> RDY state).

5. Check for leaks on floor of instrument.

6. Check syringes for leaks and crystalline deposits.

7. Check computer boards - toggle switches should point to the back of instrument except for the front two switches on WA/TR/DIL board which point left.

Plus LYTES Option: Operating instructions, Section 6, Vol. II, should be read before the operation of the instrument.

8. Check that there is adequate NaK Std A (one-half bottle) and B (one-fourth bottle) for the day's run. If supply is low, do not pour over but replace with <u>new</u> bottle

2.5.2 Pre-Analysis - Instrument is in RDY State

1. While the instrument is going to the RDY state, check the Pneumatic and Hydraulic Supply gauges (during priming). Record values on maintenance chart.

plus LYTES:

2. Perform the RINSE function.

3. Perform the SAMPLE POSITIONING function. The test dye should be visible in the tubing at least one inch, but not more than 2 1/2 inches above the electrode housing and at least two inches in the tubing below the housing. If not, adjust the value in the SAMPLE POSITIONING field, then recheck.

4. Request calibration.

2.5.3 Sample Analysis - Numbers in parenthesis indicate the section and page of DACOS Manual.

1. Check reagent volumes and replenish if needed.

2. Implement test request program for the specimen.

   a. Enter the numeric part of the ARIC identification number (NNNNNN) as the lab identification number.
   b. Enter the ARIC identification number (ANNNNNN) as the patient identification number.
   c. Enter the Batch Number as the patient name.
   d. Use profile number 928 to enter the ARIC battery of tests.

3. Do NaK bleach and calibrate.

4. Load dummy calibration tray with calibrators and controls. Recalibration is usually needed after a reagent change. Run controls on all ARIC tests and calibrate only those tests which need recalibration. Place tray on analyzer.

5. Change instrument state from Ready to Active (sample analysis). Request appropriate operation to start calibration and control analysis (F2).

6. Verify that all controls and calibrations are within range. Print out the Quality Control report for the controls. Cross out the tests that are not in the ARIC battery, staple the pages together, and file them in the black notebook for controls.

7. Load the sample tray with samples per work list assignment (6.26). Place sample tray on analyzer. The batch can be split between two trays if there are several specimens, to ensure having enough reagent for all samples.

8. Change instrument state to ACTIVE (sample analysis) (6.26). Request appropriate operation to start analysis (F1).

9. At the beginning of the run, check that the wash probes and sample and reagent arms are operating properly.

2.5.4 Data Review (6.34)

1. Access the calibration and control menus to review calibrator and control results.

2. Use the Demand Calibration and/or the Demand Control procedure (see Sec. 6.32 and 33 of DACOS Manual) to repeat a bad calibration(s) and/or control(s).

3. Print out control results.

4. Record specimen appearance and transport condition on DACOS result printouts by using the following codes:

Specimen Appearance		Transport Condition	
10	normal	00	frozen and unbroken
11	hemolyzed	01	frozen, but vial broken
12	icteric	02	thawed in transit, vial unbroken
13	slightly lipemic, specimen not ultracentrifuged	03	thawed in transit, vial broken
14	hemolyzed and lipemic		
15	icteric and lipemic		
16	lipemic, specimen ultracentrifuged		

5. Record alert values on call board. Include specimen number, batch number, test, and test value. Also record any other abnormal results on participants with alert values.

2.5.5 Shutdown (6.35 and 3.9 of Plus Lytes Manual)

1. Perform NaK maintenance after final STBY. (Bleach and wash procedure.)

2. Print cuvette #1 raw data.

3. Print requested tests (workload summary).

4. Print and purge ATTN Q.

5. Transfer QC data.

6. Purge sample trays. Be sure all patient results have been printed.

7. Change instrument state from READY to WARM.

8. Request instrument shutdown.

9. Check reagents; refrigerate the uric acid reagent and the uricase. Also refrigerate the α keto and triglyceride reagents if they are still in the carousel (from previous DACOS user). Discard reagents which are good for only one day, and leave remaining reagents in reagent carousel.

10. Leave control menu on screen.

2.6 Algorithm Processing

The DACOS analyzer is programmed to process data collected on samples, calibrators and controls through a series of data processing levels. Each of these levels has several optional methods to collect or process the data. The selection of the various options becomes part of the test definition for that procedure. The DAR reagent's test definitions are preprogrammed into the DACOS analyzer.

The collective term for these data processing levels is algorithm processing (ALG PROC). An algorithm is a series of calculations that converts raw data into a corrected result. Algorithm processing is divided into five distinct levels: raw data processing, preprocessing, main algorithm, postprocessing, calibration and result calculation. All data, whether from a sample, control or calibrator, must proceed through each level before a result is produced and a test result is printed on the Patient Report. The data processing can be made specific for any of the following types of reactions: endpoint, linear rate, initial rate, or exponential.

1. Endpoint. This is also known as an equilibrium reaction. An endpoint reaction is one in which the final absorbance change achieved at reaction completion has a direct relationship to the concentration of the substance being measured.

2. Linear Rate. This is also known as a zero-order kinetic reaction. A linear rate reaction is one in which the rate of absorbance change with time is constant and has a direct relationship to the activity of the substance being determined. This is most often used in enzyme test methodologies.

3. Initial Rate. An initial rate reaction is one in which the rate of change in absorbance has a direct relationship to the concentration of the substance being measured.

4. Exponential. This is also known as a first-order kinetic reaction. An exponential reaction is one in which the nonlinear change in absorbance with time has a direct relationship to the concentration of the substance being determined.

The calculations and data processing are individualized for each sample and test method.

2.7 Results

2.7.1 The DACOS prints specimen results in concentration.

2.7.2 Reference ranges and reporting format are in Table 1.

2.7.3 The linear range for each determination is shown below:

Creatinine	0-25 mg/dL
Glucose	0-600 mg/dL
Magnesium	0-3.5 meq/L
Potassium	0.5-10.0 mmol/L
Sodium	50-200 mmol/L
Uric Acid	0-10 mg/dL

2.7.4 Control ranges (taken from control charts in use in CCL) and calibrators are programmed into the DACOS, and unacceptable results are flagged with the code ATTQ (attention queue) on the DACOS screen. Out of range values are repeated and results evaluated. Results are screened to ensure that dilutions are made when appropriate and that a duplicate determination is run on specimens with low values. Specimens that have values above the linear range for the analyte in question are diluted with an equal volume of DACOS diluent. These activities are performed during DATA REVIEW.

2.7.5 Significant abnormalities in certain of the chemical tests in the ARIC protocol require notification to the appropriate field center. (See section 1.6 and 1.7.)

2.8 Maintenance

See pages 3.2 through 3.21 of DACOS manual for maintenance procedures.

2.9 References

1. DACOS Analyzer Reference Manual, Maintenance, Volume 1, 4235543.

2. DACOS Analyzer Reference Manual, Volume 2, 4235543.

3. DACOS Analyzer Reference Manual, VDT Program, Volume 3, 4235543.

4. DACOS Analyzer plus LYTES Option, Manual 4235473 B/February 1985.

3. CREATININE

3.1 Introduction

Creatinine is the waste product derived from creatine and is released into the plasma at a relatively constant rate. Creatinine and urea are excreted by the kidneys and are measured primarily to assess renal function.

3.2 Principle

The method used by the DART Creatinine reagent is a modified kinetic Jaffe method (3). The creatinine reacts with picrate in an alkaline solution to form a red creatinine-picrate complex, which is proportional to the amount of creatinine present. The concentration is measured at 520 nm.

3.3 Reagents

3.3.1 Water (NCCLS Type II)

Service Deionization System (SDI). The SDI process consists of a 5 micron prefilter followed by one activated carbon and two ion exchange tanks in series. This meets NCCLS Type II water requirements (Note 3).

3.3.2 DART Creatinine A+B Reagent

Manufactured by Coulter Diagnostics, a division of Coulter Electronics, Inc., Hialeah, FL. Coulter product #7546058. CMS Product #090-522. A package contains: Reagent A, ten 18 ml bottles; Reagent B, ten 15.2 ml bottles.

Store unopened DART Creatinine reagent vials at a controlled room temperature in subdued light. DO NOT REFRIGERATE. If exposed to extreme cold, crystals may appear; allow to stand at room temperature with occasional mixing to dissolve crystals. Refer to the expiration dates listed on the vial labels for shelf life. Reagent is stable for at least 72 hours at 15°C after being opened, but should be kept tightly capped when not in use.

Active Ingredients:
The values listed are the working concentrations obtained after the reagents are diluted by the DACOS Chemistry Analyzer.

Reagent A	Concentrations
Sodium Hyroxide	239.2 µmol/L
Sodium Borate	13.2 mmol/L
Sodium Phosphate Dibasic	14.1 mmol/L
Stabilizer and Surfactant	

Reagent B	Concentrations
Picric Acid	11.1 mmol/L

Reagent Preparation:
Reagent A and B are in ready-to-use form. If a problem is encountered with a new lot number, see Note 2.

Caution: Reagent A contains sodium hydroxide which may cause irritation or burns to skin and eyes. Flush contact surface with water. If taken internally or eye contact occurs, consult a physician. Use with adequate ventilation. Reagent B contains picric acid; contact with skin should be avoided. Flush contact surface with water. If Reagent is permitted to evaporate to dryness, the resulting crystals are explosive. Wash all containers thoroughly before allowing to dry.

3.3.3 DACAL Calibrators (I, II, III)

Manufactured by Coulter Diagnostics, a division of Coulter Electronics, Inc., Hialeah, FL. Coulter product #7546699. CMS product #135-145.

For this procedure, four levels of calibrators are used in duplicate:

Calibrator 1	water	0
Calibrator 2	DACAL I	1.2 mg/dL
Calibrator 3	DACAL II	3.0 mg/dL
Calibrator 4	DACAL III	5.6 mg/dL

3.3.4 Control

Two levels of clear liquid control are used. See current control charts for control in use.

3.4 Procedure

3.4.1 The creatinine test parameters are listed in Note 1.

3.4.2 See section 2 for instrument set-up.

3.4.3 Follow the DACOS general operator instruction to request, set up tray, and start measurement.

3.4.4 Four calibration points, shown above, are used to set the curve.

3.5 Calculation

3.5.1 Data Processing Steps

1. Preprocessing: outlier rejection
2. Main algorithm: initial rate
3. Postprocessing: none
4. Calibration method: regression with reagent blank.

3.5.2 The sample results are calculated by taking the final absorbances from postprocessing, subtracting the intercept of the calibration data, and then dividing by the slope of the calibration curve.

R = absorbance
A = intercept
B = slope of calibration curve

$$\frac{R - A}{B} = mg/dl$$

3.6 Results

See Section 2.7.

3.7 Notes

1. The following parameters are programmed into the DACOS for the creatinine analysis. These parameters have been researched and evaluated. They should not be adjusted. (A complete listing may be obtained using the Test Definition program.)

TEST ID:	007
Temperature:	37°C
SAMPLE VOLUME:	20 µl
DILUENT VOLUME:	120 µl
NUMBER OF REAGENTS:	2
CAROUSEL:	Both inner and outer
REAGENT DELIVERY VOLUME:	(A) 80 µL, (B) 80 µl
WAVELENGTH:	520 nm
REACTION TYPE:	Initial Rate
NO. OF DATA POINTS:	8

2. Do not use if the reagent blank has an absorbance greater than 0.060 measured at 520 nm. The reagent blank absorbance of a mixture of 800 µL of reagent A, 800 µl of reagent B, and 1.2 ml of DACOS DILUENT may be measured in a spectrophotometer against water in a 1 cm light path cuvet. This reagent blank absorbance check is an independent procedure. Therefore, its limits are not always identical to the absorbance limits of the reagent blank (first level calibrator) which are programmed in the test definition. This check is provided as a means to separate reagent problems from instrument problems.

3. NCCLS Type II water is different from NCCLS Type I in that it does not use the 0.22 micron filter to remove particulate matter greater than 0.2 microns.

3.8 References

1. DART CREATININE A + B package insert, PN 7507440F R11-84. Coulter Electronics, Inc., Hialeah, Fl.

2. DACAL CALIBRATORS (I, II, III) package insert, PN 7507445C R1-83.

3. Lustgarten JA, Wenk RF. Simple rapid kinetic method for serum creatinine measurement. Clin Chem 1972; 18(11): 1419-1422.

4. GLUCOSE

4.1 Introduction

Glucose is the transport form of carbohydrate in the body and an important energy source for all cells. Ingested starches and sugars are converted to glucose by digestive enzymes and by the liver. When dietary sources of glucose are not available, the liver synthesizes glucose from glycogen and protein. The most common disease related to carbohydrate metabolism is diabetes. The measurement of glucose is used in the diagnosis and treatment of diabetes and other states of hyper- or hypoglycemia.

4.2 Principle

Hexokinase (HK) catalyzes the reaction of glucose with adenosine-5'-triphosphate (ATP) to produce D-glucose-6-phosphate and adenosine-5'-diphosphate (ADP). D-glucose-6-phosphate is then oxidized by glucose-6-phosphate dehydrogenase (G-6-PD) to 6-phosphogluconate with the reduction in nicotinamide adenine dinucleotide phosphate (NADP+). This increase in absorbance at 340 nm is proportional to the glucose concentration in the sample.

$$\text{D-glucose} + \text{ATP} \xrightarrow[\text{HK}]{\text{Mg++}} \text{D-Glucose-6-Phosphate} + \text{ADP}$$

$$\text{D-glucose-6-Phosphate} + \text{NADP} + \xrightarrow{\text{G-6-PD}} \text{6-Phosphogluconate} + \text{NADPH} + \text{H}^+$$

The optimized DART GLUCOSE reagent method is a modified Hexokinase/Glucose-
6-Phosphate Dehydrogenase procedure, a CDC National Glucose Reference method (3).

4.3 Reagents

4.3.1 Water (NCCLS Type II)

Service Deionization System (SDI). The SDI process consists of a 5 micron prefilter followed by one activated carbon and two ion exchange tanks in series. This meets NCCLS Type II water requirements (Note 3).

4.3.2 DART Glucose A+B Reagent

Manufactured by Coulter Diagnostics, a division of Coulter Electronics, Inc., Hialeah, FL. Coulter product #7546860. CMS Product #249-443. A package contains twenty 24 ml bottles.

Store unopened DART GLUCOSE reagent vials in subdued light at 2 to 8°C. Refer to the expiration date on the vial label for shelf life. The reconstituted reagent is stable for at least 96 hours at 2 to 8°C, or 72 hours at 15°C. Do not use if the dry reagent appears most or if the reconstituted reagent becomes turbid.

Active Ingredients:
The values listed are the working concentrations obtained after the reagent is diluted by the DACOS Chemistry Analyzer.

Magnesium (Mg++)	5.0 µmol/L
Adenosine-5'-Triphosphate (ATP)	900 µmol/L
Oxidized Nicotinamide Adenine Dinucleotide Phosphate (NADP+)	900 µmol/L
Hexokinase (E.C.2.7.1.1, HK)	900 U/L
Glucose-6-Phosphate-Dehydrogenase (E.C.1.1.1.49, G-6-PD)	900 U/L
Tris (Hydroxymethyl) Aminomethane Buffer	100 mmol/L
Stabilizers	

Reagent Preparation:

Gently tap each vial of DART GLUCOSE reagent several times to loosen contents from sides of container. Add 24.0 mL of water which meets or exceeds the NCCLS specifications for Type II water. Immediately mix by gently swirling and inverting to avoid foaming, until contents are completely dissolved. If a problem is encountered with a new lot number, see Note 2.

4.3.3 DACAL Calibrator I, II, III

Manufactured by Coulter Diagnostics, a division of Coulter Electronics, Inc., Hialeah, FL. Coulter product #7546699. CMS product #135-145.

For this procedure, four levels of calibrators are used in duplicate:

Calibrator 1	water	0
Calibrator 2	DACAL I	70 mg/dL
Calibrator 3	DACAL II	204 mg/dL
Calibrator 4	DACAL III	378 mg/dL

4.3.4 Control

Two levels of clear liquid control are used. See current control charts for controls in use.

4.4 Procedure

4.4.1 The glucose test parameters are listed in Note 1.

4.4.2 See Section 2 for instrument set-up.

4.4.3 Follow the DACOS general operator instruction to request, set up tray, and start measurement.

4.4.4 Four calibration points (as shown above) are used to set the curve.

4.5 Calculation

4.5.1 Data Processing Steps

1. Preprocessing: outlier rejection
2. Main algorithm: endpoint
3. Postprocessing: monochromatic sample blank
4. Calibration method: regression with reagent blank.

4.5.2 Calculation

The sample results are calculated by taking the final absorbances from postprocessing, subtracting the intercept of the calibration data, and then dividing by the slope of the calibration curve.

R = absorbance
A = intercept -
B = slope of calibration curve

$$\frac{R - A}{B} = \text{mg/dl}$$

4.6 Results

See section 2.7.

4.7 Notes

1. The following parameters are programmed into the DACOS for the glucose analysis. These parameters have been researched and evaluated. They should not be adjusted. (A complete listing may be obtained using the Test Definition program.)

TEST ID:	006
Temperature:	37°C
SAMPLE VOLUME:	3 µl
DILUENT VOLUME:	120 µl
NUMBER OF REAGENTS:	1
CAROUSEL:	outer
REAGENT DELIVERY VOLUME:	200 µl
WAVELENGTH:	340
REACTION TYPE:	endpoint
NO. OF DATA POINTS:	20

2. Do not use if the dry reagent appears moist or if the reconstituted reagent becomes turbid. Do not use if the reagent blank has an absorbance greater than 0.150, measured at 340 nm. The reagent blank absorbance of a mixture of 2.0 mL reconstituted reagent and 1.5 mL DACOS DILUENT may be measured in a spectrophotometer against water in a 1 cm light path cuvet. This reagent blank absorbance check is an independent procedure. Therefore, its limits are not always identical to the absorbance limits of the reagent blank (first level calibrator) which are programmed in the test definition. This check is provided as a means to separate reagent problems from instrument problems.

   Inability to recover results within the expected ranges of the controls in use while using DACAL I, II, and III calibrators to calibrate may indicate reagent deterioration.

3. NCCLS Type II water is different from NCCLS Type I in that it does not use the 0.22 micron filter to remove particulate matter greater than 0.2 microns.

4.8 References

1. DART GLUCOSE package insert, PN 750779A R5-85. Coulter Electronics, Inc., Hialeah, FL.

2. DACAL CALIBRATORS package insert, PN 7507445C R1-83. Coulter Electronics, Inc., Hialeah, FL.

3. Publication No. (CDC) 77-8330, U.S. Department of Health, Education, and Welfare, 1976. National Glucose Reference Method.

5. MAGNESIUM

5.1 Introduction

Magnesium is, next to potassium, the most prevalent intracellular cation. Approximately 65-70% of magnesium in blood is in the diffusible or free form; the remainder is protein bound (primarily to albumin). Magnesium serves as an activating ion in enzyme systems involved in lipid, carbohydrate, and protein metabolism. Magnesium deficiency causes irritability of the nervous system with tetany, vasodilation, convulsions, tremors, depression, and psychotic behavior. High levels reduce muscle and nerve irritability and can result in anesthesia (loss of sensations of touch, temperature, pain) and cardiac arrest.

5.2 Principle

The measurement of magnesium is based on the procedure of Gindler and Heth (2) which uses the metallochromic dye, Calmagite [1-(1-Hydroxy-4-methyl-2-phenylazo) -2-napthol-4-sulfonic acid]. The working reagent contains Calmagite, which is bound to a mixture of the 9-ethyleneoxide adduct of p-nonylphenol and polyvinylpyrrolidone. These latter materials prevent interference by proteins which would otherwise alter the spectrum of Calmagite. Potassium hydroxide keeps the calmagite as the blue alkaline ionic species. The addition of magnesium salts causes the formation of the red magnesium complex of calmagite. Calcium interference is virtually eliminated by preferential combination with ECTA [ethylenebis (oxyethyl-enenitrilo) tetraacetic-acid], which is present in the system in the form of a salt. Interference by heavy metals is prevented by the presence of cyanide. The red magnesium calmagite complex is measured at 532 nm.

5.3 Reagents

5.3.1 Water (NCCLS Type II)

Service Deionization System (SDI). The SDI process consists of a 5 micron prefilter followed by one activated carbon and two ion exchange tanks in series. This meets NCCLS Type II water requirements (Note 2).

5.3.2 Lancer Magnesium Reagents

Manufactured by Lancer Division of Sherwood Medical. Product number for base is 8888-455043. Product number for dye is 8883-455084. These bulk reagents are stable at room temperature (18-25°C) at least until expiration date shown on the label.

1.	Dye Reagent	
	Calmagite	0.06 g/L
	Potassium Chloride	28 g/L
	BionM NE-9	1.08 g/L
	Bion PVP	10 g/L
2.	Base Reagent	
	Potassium Cyanide	2.0 g/L
	Potassium Hydroxide	15.8 g/L
	EGTA	0.45 g/L

Reagent Preparation: The working reagent is prepared by mixing ten volumes of Dye Reagent with one volume of Base Reagent. The working reagent is stable 24 hours at room temperature (18-25°C).

Caution: The Magnesium Base Reagent is both poisonous and caustic. Care must be taken in handling and disposing of this reagent.

Dye Reagent contains 1.25 g/L sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

5.3.3 Calibrators

Aqueous standards made in-house are used to calibrate the magnesium procedure. The standards also contain calcium. Instructions on making the stock and working standards recorded below are from the procedure for calcium and magnesium on the Atonomic Absorption Spectrophotometer (Instrumentation Laboratory Model Video 12 aa/ae). The standards made for the Atomic Absorption Spectrophotometer are shared with the DACOS.

Stock Standards:

Calcium - 100 mg/dL. Weigh 2.4973 gm - pulverized and dessicated $CaCO_3$ Iceland Spar on Baker #4918 or use NBS Standard Reference Material 915. Dissolve in 125 mL 1 N HCl. Dilute to 1 liter with Milli-Q water. Store in pyrex container. The calcium stock standard is stable for one year at room temperature.

Magnesium - 100 meq/L. Dissolve 1.216 gm of pure magnesium metal in 9 to 10 mL of 12 N HCl and dilute to 1 liter with Milli-Q water. Store in pyrex bottles. The magnesium stock standard is stable for one year at room temperature.

Intermediate Standard:

Intermediate Ca-Mg standards (2-0.5; 5-1; 8-2; 10-3; 12-3.5; 15-4 mg/dLmeq/L): To 200 mL volumetric flasks, add the respective amounts of calcium and magnesium stock standards and dilute to volume with Milli-Q water. Store in polypropylene bottles. Make up two 200 mL volumetrics of the 8 mg/dL-2 meq/L standard. The mg/dL-0.5 meq/L standard is only used when needed. The intermediate standards are stable for six months at room temperature.

Concentration of Inter. Std CA-MG mg/dL - meq/L)	ML of Stock Std CA-MG (100 mg/dL-100 meq/L)	Diluted to volume with Milli-Q water
2 - 0.5	4.0 - 1.0	200 mL
5 - 1	10.0 - 2.0	200 mL
8 - 2	16.0 - 4.0	200 mL
10 - 3	20.0 - 6.0	200 mL
12 - 3.5	24.0 - 7.0	200 mL
15 - 4	30.0 - 8.0	200 mL

Store the standards in polypropyle bottles. The standards are stable for six months at room temperature.

Working Standards (Calibrators):

The 3 meq/L magnesium standard from the AAS procedure is diluted 1:2 to obtain a 1.5 meq/L standard. The 3 meq/L standard is also diluted 1:5 to obtain a 0.6 meq/L standard. For this procedure, four levels of calibration are used:

Calibrator 1	water	0
Calibrator 2	standard	0.6 meq/L magnesium
Calibrator 3	standard	1.5 meq/L magnesium
Calibrator 4	standard	3.0 meq/L magnesium

Procedure to Check New Standards:

a. Prepare working dilutions of the old intermediate standards as usual.
b. Prepare working dilutions of the new intermediate standards with the 8 mg/dL - 2 meq/L standard prepared in duplicate.
c. Set up the calcium and magnesium standard line using the old working standards. Read the concentration of the old 8 mg/dL - 2 meq/L standard followed by the new 8 mg/dL - 2 meq/L standard twice (e.g., old 8 mg/dL - 2 meq/L, new 8 mg/dL - 2 meq/L, old, new).
d. Calculate the % accuracy:

$$\frac{\text{Average reading of new 8 mg/dL - 2 meq/L std}}{\text{Average reading of old 8 mg/dL - 2 meq/L std}} \times 100 = \% \text{ accuracy}$$

The accuracy should be 99-101%.
e. Read the concentrations of the remaining new standards to check that they are within acceptable range of ± 0.15 for calcium standards and ± 0.10 for magnesium standards and that linearity is present.
f. Check new stock standards on two days. Check new intermediate standards made from a current stock as described in Step e.

5.3.4 Control

A clear liquid control is used. See current control charts for control in use.

5.4 Procedure

5.4.1 The magnesium test parameters are listed in Note 1.

5.4.2 See section 2 for instrument set-up.

5.4.3 Follow the DACOS general operator instruction to request, set up tray, and start measurement.

5.4.4 Three calibration points, shown above, are used to set the curve.

5.5 Calculation

5.5.1 Data Processing Steps

1. Preprocessing: outlier rejection
2. Main algorithm: endpoint reaction
3. Postprocessing: monochromatic sample blank
4. Calibration method: regression with reagent blank.

5.5.2 Calculation

The sample results are calculated by taking the final absorbances from postprocessing, subtracting the intercept of the calibration data, and then dividing by the slope of the calibration curve.

R = absorbance
A = intercept
B = slope of calibration curve

$$\frac{R - A}{B} = \text{mg/dl}$$

5.6 Results

See section 2.7.

5.7 Notes

1. The following parameters are programmed into the DACOS for the magnesium analysis. These parameters have been researched and evaluated. They should not be adjusted. (A complete listing may be obtained using the Test Definition program.)

TEST ID:	034
Temperature:	37°C
SAMPLE VOLUME:	4 µl
DILUENT VOLUME:	120 µl
NUMBER OF REAGENTS:	1
CAROUSEL:	inner
REAGENT DELIVERY VOLUME:	200 µl
WAVELENGTH:	520
REACTION TYPE:	End Point
NO. OF DATA POINTS:	30

2. NCCLS Type II water is different from NCCLS Type I in that it does not use the 0.22 micron filter to remove particulate matter greater than 0.2 microns.

5.8 References

1. Lancer Magnesium Rapid Stat Diagnostic Kit package insert 021-051 for Lancer Division of Sherwood Medical, 1960.

2. Grindler EM, Heth DA. Clin Chem 1971; 17:662.

6. POTASSIUM

See Sodium/Potassium procedure 7.

7. SODIUM/POTASSIUM

7.1 Introduction

Sodium is the major cation of extra cellular fluid. Potassium is the major intracellular cation. Abnormal sodium and potassium levels can cause disturbances in several organs.

7.2 Principle

The measurement of sodium and potassium is done by an ion selective electrode (ISE) in the plus LYTES Option. The ISE performs a direct electrochemical measurement on undiluted serum. The millivolt responses of the sodium and potassium electrodes are logarithmically proportional to sodium or potassium ion activity in the serum.

An ion-selective electrode as well as a reference electrode are needed for this measurement. The sodium electrode is a glass membrane type. It is specifically formulated for high selectivity and sodium ions. The reference electrode is of the AG/AgCl type. The potassium electrode system is similar except that valinomycin, an antibiotic, is used for the membrane. Valinomycin is able to replace the hydration shell of a potassium cation thereby making the ISE highly selective for potassium.

7.3 Reagent

7.3.1 Water (NCCLS Type II)

Service Deionization System (SDI). The SDI process consists of a 5 micron prefilter followed by one activated carbon and two ion exchange tanks in series. This meets NCCLS Type II water requirements (Note 2).

7.3.2 Calibrators

DART Na/K Standard Solution A: This solution is required to produce one level of the two-point calibration done for both potassium and sodium. It is also used to produce the one-point calibration done with each sample analysis. It is pumped from its storage bottle to the standard A delivery station where it is aspirated by the electrolyte probe during the calibration function.

Package contents:	4 x 330 mL	
Concentration:	Sodium (Na+)	140.0 mmol/L
	Potassium (K+)	4.0 mmol/L
Ingredients:	Sodium Chloride	137.0 mmol/L
	Potassium Chloride	4.0 mmol/L
	Sodium Phosphate, Monobasic	3.0 mmol/L
	Wetting Agent	

DART Na/K Standard Solution B: This solution is required to produce one level of the two-point calibration done for both potassium and sodium. It is pumped from its storage bottle to the standard B delivery station where it is aspirated by the electrolyte probe during the calibration function which occurs every two hours.

Package contents:	4 x 330 mL	
Concentration:	Sodium (Na+)	80.0 mmol/L
	Potassium (K+)	16.0 mmol/L
Ingredients:	Sodium Chloride	77.0 mmol/L
	Potassium Chloride	16.0 mmol/L
	Sodium Phosphate, Monobasic	3.0 mmol/L
	Wetting Agent	

7.3.3 Controls

Two levels of clear lyophilized control are used to set the curve. See current control charts for control in use.

7.4 Procedure

7.4.1 The sodium/potassium test parameters are listed in Note 1.

7.4.2 See section 2.8 for instrument set-up.

7.4.3 Follow the DACOS general operator instruction to request, set-up tray, and start measurement.

7.5 Calculation

A two-point calibration is performed using standards A and B. The slope of the calibration curve is substituted in the Nernst equation which is then used to calculate the sample sodium or potassium concentration. There is a further algebreic correction to the sodium and potassium concentration to correlate the results to other (flame and photometry and indirect ISE on the ASTRA) instrumentation.

The following factors are programmed into the DACOS to accomplish that correlation:

$$[\text{Sodium}]_{\text{reported}} = 0.98 \times [\text{Sodium}]_{\text{uncorrected direct ISE}}$$

$$[\text{Potassium}]_{\text{reported}} = 0.96 \times [\text{Potassium}]_{\text{uncorrected direct ISE}}$$

These correction factors are necessary to account for binding of the ions by plasma proteins, residual liquid-junction potentials, the water content of the serum, and activity coefficients for sodium and potassium ions in plasma.

7.6 Results

See section 2.7.

7.7 Notes

1. The following parameters are programmed into the DACOS for sodium and potassium. These parameters have been researched and evaluated. They should not be adjusted.

	Sodium	Potassium
Test ID	001	002
Temperature	37°	37°
Sample Volume	175 µL*	175 µL*
Reaction type	Direct ISE	Direct ISE
Flame photometer equivalence factor	0.98	0.96

*175 µL of specimen is used for both Na and K.

2. NCCLS Type II water is different from NCCLS Type I in that it does not use the 0.22 micron filter to remove particulate matter greater than 0.2 microns.

7.8 References

1. DACOS Analyzer plus LYTES Option Manual. PN 6602743.

2. Fuhrman SA, Eckfeldt JH. Hyponatremia and ion-selective electrodes. Ann Intern Med 1985; 102:872, Letter.

3. Miller WG. Sodium and potassium. In: Kaplan L, Pesce A (eds), Clinical chemistry: theory, analysis, and correlation. St. Louis: C.V. Mosby Co, 1984; 1075-8.

4. Mass AHJ, Siggaard-Anderson O, Weisberg HF, Zulstra W. Ion-selective electrodes for sodium and potassium: a new problem of what is measured and what should be reported. Clin Chem 1985; 31:482-485.

8. URIC ACID

8.1 Introduction

Uric acid is the end product of purine metabolism in man. Plasma uric acid is filtered by the glomeruli and about 90% is reabsorbed by the tubules. The serious consequences of abnormal uric acid metabolism is dependent on the insolubility of uric acid and sodium mono-urate which either crystallize in the kidney and urinary tract or in the cartilage and tissues around the joints in gout.

Increased uric acid levels are found primarily in gout, excessive nucleo-protein metabolism (such as leukemia and cancer chemotherapy) and in renal failure. A purine-rich diet (liver, kidney, sweetbread) may increase the uric acid level.

Decreased uric acid levels are rare, but are found in Wilson's disease (hepatolenticular degeneration), Fanconi syndrome (a congenital tubular defect) and xanthine oxidase deficiency. Uricosuric drug therapy promotes the excretion of uric acid by the kidney as treatment to lower blood uric acid levels. Allopurinol is often used as it is a competitive inhibitor of xanthine oxidase and causes a fall in uric acid by blocking its synthesis from the purines xanthine and hypoxanthine.

8.2 Principle

Uric acid is oxidized in the presence of uricase. The hydrogen peroxide produced reacts with ethanol in the presence of catalase to produce acetaldehyde. The acetaldehyde is oxidized to acetate in the reaction catalyzed by aldehyde dehydrogenase (ALDH). The increase in absorbance at 340 nm produced by NADP reduction is proportional to the amount of uric acid in the sample. The reagent system is based on the method of Haeckel (2).

$$\text{Uric Acid} + O_2 + 2\,H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + H_2O_2 + CO_2$$

$$H_2O_2 + \text{Ethanol} \xrightarrow{\text{Catalase}} 2H_2O + \text{Acetaldehyde}$$

$$\text{Acetaldehyde} + NADP^+ + \xrightarrow[\text{Dehydrogenase}]{\text{Aldehyde}} NADPH + H^+ + \text{Acetate}$$

8.3 Reagents

8.3.1 Water (NCCLS Type II)

Service Deionization System (SDI). The SDI process consists of a 5 micron prefilter followed by one activated carbon and two ion exchange tanks in series. This meets NCCLS Type II water requirements (Note 3).

8.3.2 Dri-STAT Uric Acid-UV Endpoint Reagent

Manufactured by Beckman Instruments, Inc., Carlsbad, CA. Beckman reorder #683392. A package contains the following:

Reagent - 10 containers, 10 mL each when reconstituted
Diluent - 1 container, 200 mL
Uricase - 3 containers, 1.5 mL each when dissolved

The unopened reagent components are stable until the expiration date stated on the labels when stored as recommended. Diluent should be stored at 20°C to 25°C; reagent and uricase at 2°C to 8°C. The values listed below for the active ingredients are the concentrations in the components as formulated.

1. Diluent:
   Ethanol 1.09 mol/L
   Glycerol 0.34 mol/L
   Diluent must be at 20°-25°C before use

2. Reagent:
   NADP 1.5 mmol/L
   Catalase (animal) 1330 KU/L
   ALDH (microbial) 380 U/L
   Buffer, pH 8.5 ± 0.1

   Add 20 mL of diluent to each reagent vial. After the reagent is dissolved, invert to mix. After reconstitution, reagent is stable for 8 hours at room temperature or 36 hours at 2°-8°C. See Note 2.

3. Uricase:
   Uricase (microbial) 20 KUl

   Add 1.5 mL of NCCLS Type II water to each uricase vial. Uricase solutions are stable 1 month at 2°-8°C. Keep refrigerated at all times prior to making working reagent.

4. Working reagent:
   Add 0.4 mL uricase solution to dissolved contents of reagent vial. Invert gently 4-5 times (DO NOT SHAKE), and wait 5 minutes before use. Uricase activity is very dependent on the mixing of the working reagent. The enzyme can be destroyed by vigorous mixing which will result in improper uricase activity.

8.3.3 Calibrators

Manufactured by Coulter Diagnostics, a division of Coulter Electronics, Inc., Hialeah, FL. Coulter prodct #7546699. CMS product #135-145.

The Uric Acid procedure is not a Coulter method. The Coulter values assigned to the calibrators cannot be used. the UMH laboratory has performed correlation studies with another UMH laboratory uric acid method that is standardized against aqueous standards and assigned values to the calibrators. See section 15.3.2 for further information.

For this procedure, four levels of calibrators are used in duplicate:

Calibrator 1	water	0 mg/dL
Calibrator 2	DACAL I	3.6 mg/dL
Calibrator 3	DACAL II	7.6 mg/dL
Calibrator 4	DACAL III	10.4 mg/dL

8.3.4 Control

Clear liquid control is used. See current control charts for control in use.

8.4 Procedure

8.4.1 The uric acid test parameters are listed in Note 1.

8.4.2 See section 2 for instrument set-up.

8.4.3 Follow the DACOS general operator instruction to request, set up tray, and start measurement.

8.4.4 Four calibration points, shown above, are used to set the curve.

8.5 Calculation

8.5.1 Data Processing Steps

1. Preprocessing: outlier rejection
2. Main algorithm: endpoint
3. Postprocessing: monochromatic sample blank
4. Calibration method: regression with reagent blank.

8.5.2 Calculation

The sample results are calculated by taking the final absorbances from postprocessing, subtracting the intercept of the calibration data, and then dividing by the slope of the calibration curve.

R = absorbance
A = intercept
B = slope of calibration curve

$$\frac{R - A}{B} = \text{mg/dl}$$

8.6 Results

See section 2.7.

8.7 Notes

1. The following parameters are programmed into the DACOS for the uric acid analysis. These parameters have been researched and evaluated. They should not be adjusted. (A complete listing may be obtained using the Test Definition program.)

TEST ID:	008
Temperature:	37°C
SAMPLE VOLUME:	20 µl
DILUENT VOLUME:	40 µl
NUMBER OF REAGENTS:	2
CAROUSEL:	inner and outer
REAGENT DELIVERY VOLUME:	inner = 20 µl; outer = 250 µl
WAVELENGTH:	340 nm
REACTION TYPE:	endpoint
NO. OF DATA POINTS:	22

2. A flocculent precipitate may occasionally form in the reagent component. It may be removed by filtration without altering reagent performance.

3. NCCLS Type II water is different from NCCLS Type I in that it does not use the 0.22 micron filter to remove particulate matter greater than 0.2 microns.

8.8 References

1. Dri-STAT Uric Acid-UV Endpoint Reagent package insert. Beckman instructions 015-245484, August 1983. Beckman Instruments, Inc., Fullerton, CA.

2. Haeckel R. The use of aldehyde dehydrogenase to determine H_2O_2 producing reactions. 1. The determination of the uric acid concentration. J Clin Chem Clin Biochem 1976; 14:101.

9. INSULIN

9.1 Introduction

Insulin has widespread effects on diverse cellular and subcellular processes. It facilitates amino acid movement into cells and utilization in protein synthesis, transport of sodium and potassium across cell walls, triglyceride synthesis and lipolysis. Hormonally responsive tissues include muscle (skeletal and heart), adipose, liver, white cell, mammary gland, bone, skin, lens, pituitary, peripheral nerve, and aorta. A primary role of insulin is to regulate uptake, storage, and release of circulating substrates (glucose, amino acids, and fatty acids).

Insulin levels are usually measured in conjunction with blood glucose concentrations. Maturity onset diabetes, acromegaly, glucocorticoid excess, carbohydrate deprivation, and obesity are associated with glucose intolerance and delayed hyperinsulinemia. Severe glucose intolerance and hypoinsulinemia are associated with juvenile onset diabetes, chronic pancreatitis, cystic fibrosis, and hypokalemia. In addition, spontaneous or fasting hypoglycemia associated with inappropriately high insulin concentrations is diagnostic of islet cell tumors or insulinoma.

9.2 Principle

Serum is mixed with a fixed amount of radioactively labeled insulin and with antibody to insulin. The endogenous insulin in the serum competes with the labeled insulin for antibody binding sites. Antibody-bound and unbound insulin (both endogenous and labeled) are separated by precipitating the antibody-insulin complex with antibody to gamma globulin and PEG. The radioactivity of the bound (precipitated) fraction is counted. The bound ^{125}I insulin decreases with the amount of endogenous or standard insulin in the system.

9.3 Specimen

Do not use grossly hemolyzed or grossly lipemic samples.

Optimum volume: 500 µL
Volume pipeted per assay: 200 µl

9.4 Equipment and Supplies

1. Gamma counter
2. IsoData data reduction system
3. Calibrated pipettes or automatic dilutors to deliver required volumes (50 µL, 100 µl, 200 µl, 1000 µl, and 2 mL)
4. 12 x 75 mm polystyrene assay tubes
5. Vortex mixer
6. Radioactive waste containers

7. Absorbent paper
8. Timer
9. Refrigerator and freezer
10. Linear linear graph paper
11. Deionized or distilled water
12. Refrigerated centrifuge (4°C)
13. Test tube racks
14. Foam decanting racks
15. Parafilm

9.5 Reagents

9.5.1 Cambridge ^{125}I Insulin 100 test kit, 732.

Caution: Potential source of hepatitis B - Surface antigen (HB_sAg) and/or HTLV111.

1. Kit reagents provided

   a. Assay buffer (641) - Borate buffer containing EDTA, BSA, and preservative - 25 mL/vial.
   b. Insulin antiserum (guinea pig) (230) - Guinea pig antiinsulin antiserum in assay buffer - 11 mL/vial.
   c. ^{125}I insulin (130K) - ^{125}I insulin and normal guinea pig serum in assay buffer - 11 mL/vial, approximately 1.0 µCi/vial.

Caution: Radioactive material.

   d. Precipitating reagent (688) - Goat anti-guinea pig gamma globulin and PEG in borate buffer containing EDTA and preservative - 100 mL/bottle. MIX WELL BEFORE USE.
   e. Insulin standards (330) - Porcine insulin in assay buffer at the following nominal concentrations: 5, 15, 35, 75, 150, and 300 µU/mL (mU/L) - 2 mL/vial. Standards are calibrated against the first International Reference Preparation, WHO, 1974. Use assay buffer as the zero standard.
   f. Insulin kit controls (530 and 531) - Porcine insulin lyophilized in human serum, plus preservative. Reconstitute each vial with 2 mL deionized or distilled water.

9.5.2 Controls

1. Refer to control book for information about controls currently in use.

2. Dilute and store commercial controls according to manufacturer's specifications.

3. Assay controls in duplicate at the beginning and end of each assay and also mid-batch if the assay exceeds 90 tubes.

9.5.3 Storage Instructions

1. Refrigerate all reagents at 2°-8°C. They are stable until the expiration date on the vial except for the standards and kit controls. For prolonged storage of these reagents, aliquot and freeze. Standards are stable for four months at 4°C. Kit controls are stable for eight weeks at 4°C. Both standards and kit controls are stable until the expiration date on the vial if stored frozen.

2. Prior to assay, allow all samples and reagents to equilibrate to room temperature and mix by gently swirling or inversion.

9.5.4 Reagent Precautions

1. All of the reagents except the serum controls and precipitating reagent should be clear after standing at room temperature.

2. Do not use reagents after the expiration date. Do not mix reagents from different kits unless they have the same lot number.

9.6 Instrument Set-up

See manuals and separate protocols for operation of Micromedic gamma counter, IsoData data reduction system, Beckman centrifuges (Models J-6M, TJ-R, J6, and accuspin FR), and autodilutors.

9.7 Procedure

Equilibrate all reagents and specimens to room temperature and mix before use.

1. Number duplicate 12x75 mm polystyrene test tubes for each of the following: total counts (TC), nonspecific binding (NSB), standards, controls, and specimens.

2. Pipette 200 µL assay buffer into the nonspecific binding tubes.

3. Pipette 100 µL of standards, controls, or unknown samples into the appropriate tubes.

Caution: Potential source of hepatitis B - Surface antigen (HB_sAg) and/or HTLVIII.

4. Pipette 100 µL of ^{125}I insulin into each tube. Set TC tubes aside until Step 11.

Caution: Radioactive material.

5. Pipette 100 µL of insulin antiserum into each tube except TC and NSB.

6. Vortex, cover, and incubate for 90 minutes at room temperature.

7. Shake the bottle of precipitating reagent to ensure an even suspension. Then add 1 mL to all the tubes except TC.

8. Vortex and incubate 10 minutes at RT.

9. Centrifuge for 20 minutes at 4°C, 3000 rpm.

10. Decant (except TC) and blot on absorbent paper.

11. Count each tube, including TC, for 3 minutes in a gamma counter.

12. Dispose of radioactive tubes in the appropriate metal radioactive can.

9.8 Calculation

Use the IsoData 4-PL data reduction system. For each batch of unknown samples assayed, manually plot mU/L insulin on the x axis versus percent bound on the y axis using linear linear graph paper. This manual plot is used only to observe the assay performance. Patient results are calculated by the IsoData reduction system.

9.9 Results

1. Format for reporting results:

    a. Report to whole numbers using a maximum of three significant figures.
    b. Generally, dilute values greater than 150 mU/L in the assay buffer provided. Multiply the mU/L calculated from the graph by the appropriate dilution factor to obtain the insulin concentration.
    c. The lowest reportable result is 0.0 mU/L. (The quoted sensitivity is 2.0 mU/L.)
    d. Any specimen which is to be reassayed should be put back into the workpool in the computer and a comment entered, such as ">150 mu/L to be diluted." If an ARIC speicmen is to be reassayed, record "REPEAT" on the ARIC work list.
    e. For each assay, check that the standard values recorded on the assay sheets and entered in the IsoData correspond to the values stamped on the standard vials.

2. Refer to the Cambridge ^{125}I insulin package insert and R and D book for recovery and specificity data.

9.10 References

1. Cambridge ^{125}I Insulin (732) Kit package insert, April 1984. Cambridge Medical Diagnostics, Inc., 575 Middlesex Turnpike, Billerica, MA 01865. 1-800-225-0850.

2. Manuals and procedures for operation of:
   a. Micromedic gamma counter
   b. IsoData data reduction system
   c. Beckman autodilutor
   d. Beckman centrifuges (Models J-6M, TJ-R, J6, and accuspin FR)

3. Laboratory Safety Manual - Radiation Safety

4. Crowley MF, Garbien KJT. Insulin: a comparison of the results of plasma and serum assays using a double antibody technique. Clin Chem Acta 1974; 51:345.

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10. HEMOGLOBIN A1c
GLYCOSYLATED HEMOGLOBIN

10.1 Introduction

Hemoglobin subfractions formed by the glycosylation of the alpha or beta chains of hemoglobin A (HbA) are collectively known as glycosylated hemoglobins. Hemoglobin A1c, the best-defined of these, is formed by the reversible condensation of the carbonyl group of glucose and the amino group at the N-terminus of the beta chain of hemoglobin A, resulting in a labile aldimine or Schiff base. As the red cell circulates, some of the aldimine undergoes a slow, irreversible conversion (Amadori rearrangement) to a stable ketoamine form (HbA1c). As blood glucose levels rise, the increase in glycosylated hemoglobin is proportional to both the level of glucose and the lifespan of the red cell. Therefore, the glycosylated hemoglobin result is a reflection of the mean daily blood glucose concentration and the degree of carbohydrate imbalance over the preceding two months.

10.2 Principle

Prior to analysis, an aliquot of whole blood is diluted with hemolyzing reagent and incubated at 37°C, which frees hemoglobin from the red blood cells and removes the labile Schiff base fraction. Hemolyzed samples are then placed in the autosampler compartment which is maintained at a constant temperature of 10°C, and are automatically injected onto an HPLC (High Performance Liquid Chromatography) analytical column packed with cation-exchange resin. Separation of the glycosylated fractions occurs in a timed sequence using three phosphate buffers of increasing strength. Detection occurs at 415 and 690 nm to ensure a stable baseline. Data reduction is performed by a built-in integrator which reports HbA1c and total HbA1 values and expresses relative percentages (by area) of all resolved hemoglobin subfractions.

10.3 Specimen

Optimum sample volume: 1 mL whole blood
Minimum sample volume: 50 uL whole blood

A venous whole blood specimen collected in EDTA is required. Tubes containing heparin, potassium oxalate or sodium fluoride are acceptable. Whole blood specimens can be stored up to seven days at 2-8°C, or up to 1 year frozen at -70°C. Prior to analysis, samples should be allowed to come to room temperature and gently mixed to ensure homogeneity.

10.4 Equipment and Supplies

1. DIAMAT Glycosylated Hemoglobin Analyzer (includes autodilutor). Catalog No. 196-1001, Bio-Rad Clinical Division, 1000 Alfred Nobel Drive, Hercules, CA 94547.

2. Water Bath, 37°C. Catalog No. 196-1016. Bio-Rad Clinical Division.

3. Replacement Printer Paper, pkg. of 10 rolls. Catalog No. 196-1020. Bio-Rad Clinical Division.

4. Labquake Rotator. Catalog No. 415-110. Labindustries, Inc., 620 Hearst Avenue, Berkeley, CA 94710-1992.

5. Polypropylene sample vials, 1.5 mL clear (with covers), pkg. of 1000. Catalog No. 191-2290. Bio-Rad Clinical Division. (For aliquoting reference standard for frozen storage.)

10.5 **Reagents and Supplies**

10.5.1 DIAMAT HbA1c Reorder Pack

Catalog No. 196-1005. Bio-Rad Clinical Division. Each kit contains the following reagents and supplies sufficient for 1000 tests (individual catalog numbers are provided for separate reorder):

1. HbA1c Analytical Column, 4 mm I.D. x 15 cm, includes 4 prefilters (Cat. No. 196-1013). Store at room temperature. Capable of performing 1000 analyses. Change prefilter every 250 tests. Stable until expiration date printed on label.

2. HbA1c Reference Standard Set: one 120 mL bottle of Reference Standard Diluent (KCN-borate solution) and lyophilized human whole blood hemolysate, 10 x 10 mL (Cat. No. 196-1028). Stable until expiration date printed on vial label when stored unopen at 2-8°C. Assigned reference standard value is printed on vial label. Reconstitute vial with 10 mL cold Reference Standard Diluent. Replace rubber stopper with teflon-faced cap provided. Allow vial to stand 5-10 minutes then swirl gently to dissolve. Pipette 400 uL aliquots of reference standard into labelled polypropylene vials (with covers) and store frozen at -70°C. Aliquots are stable 90 days at -70°C. Prior to analysis, thaw reference standard vials, vortex to mix thoroughly and place in refrigerated autosampler compartment. The reconstituted reference standard does not require dilution or incubation prior to analysis. Prior to putting a new lot of Reference Standard into use, dilute one vial and run aliquots in duplicate over a period of several days to verify that label value is correct.

3. Polypropylene sample vials, 1.5 mL, pkg. of 1000. (Cat. No. 196-1012).

4. Buffer set: Elution buffers 1, 2 and 3 (1 x 2 liters each), Cat. No. 196-1050, phosphate buffers containing sodium azide as preservative. Store at room temperature. Stable until expiration date printed on labels.

5. Wash solution (1 x 2 liters), Cat. No. 196-1009, phosphate buffer containing sodium azide as preservative. Store at room temperature. Stable until expiration date printed on label.

6. Hemolysis Reagent (1 x 1 liter), Cat. No. 196-1010, octylphenoxy, polyethoxyethanol (0.1% v/v) in borate buffer. Store at room temperature. Stable until expiration date printed on label.

7. Prefilters (pkg. of 10), Cat. No. 196-1015.

8. Diamat Printed Paper (10 rolls), Cat. No. 196-1020.

10.5.2 Controls

Two levels of glycosylated hemoglobin control (Normal and High) are run in duplicate with each batch. The controls are prepared from whole blood drawn from a normal (Normal) and a diabetic (High) individual. The control aliquotting procedure must be performed in a cold room. Add 150 mL of whole blood to a flask containing 4.8 mL of a solution of 8 g disodium-EDTA diluted to 100 mL in isotonic saline. Place flask on a magnetic stirrer at low speed. Aliquots of about 100 uL are pipetted into labelled polypropylene micro-centrifuge tubes with caps and stored frozen at -70°C. At the start of each week, a one week's supply of controls is taken from this stock supply and placed in the -70°C freezer containing the current supply of diluted reference standard aliquotes.

10.6 Procedure

10.6.1 Empty water bath and refill with tap water to a depth of about one inch. Turn on bath and allow to come to temperature (37°C).

10.6.2 Turn on the autodilutor. Place the inlet tubing into the hemolysis reagent reservoir and secure the cap. Remove the spacers under each syringe. Set the mode switch to CONT and tap the remote switch on the probe to initiate continuous reagent dispensing. Let syringes cycle 4-5 times to purge tubing and syringes of air and to prime the system with hemolysis reagent. When the syringes are on the upstroke, set mode switch back to MAN mode (manual dispensing). Install the 5% spacer for the 100 uL sample syringe. For the 2.5 mL reagent syringe install the 40% spacer (see Note 1). For specimens with a low hematocrit, take off specimen using the 10% and 20% sample syringe spacers. Results should agree.

10.6.3 Consult daily checklist (see example at end of procedure) prior to setting up Diamat each day. Press the POWER switch on the

operation panel to initiate a 30 minute equilibration sequence (see Note 2). Verify that parameters are set correctly by pressing F3 ENTER to generate a parameter printout and compare it to the posted example. If the main power to the Diamat has been switched off or interrupted, current parameter changes must be re-entered manually before starting the run (consult posted example of current parameter changes). After equilibration, the Diamat will enter the STANDBY mode.

10.6.4 Set up protocol sheets, starting with 3 or 4 standards. Run the controls in duplicate and select a specimen from the previous run as a between-batch duplicate (same specimen may be used for both instruments). When both Diamats are to be set up, also run a between-instrument duplicate. Place a reference standard after every 10 patient or control samples and two standards at the end of the batch. Label each sample vial (to facilitate transfer of samples between instruments if necessary) and place it in the appropriate numbered well in the metal sample rack.

10.6.5 Mix whole blood specimens on rotator for 5 minutes and vortex the thawed controls and specimens. Using the autodilutor, prepare hemolysates of patient specimens and controls by diluting 5 uL of each sample with 1.0 mL hemolysis reagent. Wipe the probe tip after drawing up sample and again after dispensing into labelled sample vial. When finished, place the metal sample rack containing the hemolysate vials into the 37°C water bath for 30 minutes.

10.6.6 When patient samples and controls have finished incubating, place the metal sample rack into the Diamat sample compartment (see Note 3), being careful not to bump the autosampler assembly. Begin the run by loading sample vials containing about 0.4 mL reconstituted HbA1c reference standard into the first 3 positions. Specify sample wells to be analyzed by entering 1st/CURR and LAST well numbers on the operation panel. Set the SAMPLE number to reflect the well from which the first injectionof the run is to be made, usually well 1. If more samples are added later, be sure to change LAST well number.

10.6.7 When START is pressed, the analyzer will equilibrate for 5 minutes, then automatically aspirate the first sample. When the autosampler probe is down in the wash well after aspiration of the sample, record the system pressure (in kg/cm^2) from the control panel data display on the daily checklist.

10.6.8 Disregard the first chromatogram. When the chromatogram for the reference standard in the next position prints out, check that the standard reads its assigned value for % HbA1c. If the % HbA1c result deviates at all from the assigned value, a new Rf (Response factor) must be programmed into the microprocessor using the keyboard on the control panel. Enter the change as follows:

F1 F1 2 F1 4 F1 4 F1 (Rf value x 100) F1 F1 F1 F1 ENTER

Press REPRINT to generate a reprint of the second standard results as recalculated with the new Rf value. Changing the Rf and reprinting standard results must be carried out before next sample report prints out (i.e. within 8 minutes). After reprinting, press F3 ENTER to generate a revised parameter printout which reflects the new Rf value. The reference standard in the following well should then read the assigned value $\pm$ 0.1, confirming that the factor has been set correctly. If not, thaw and run another standard in the STAT well and adjust factor if the result still deviates by more than $\pm$ 0.1. After initial calibration for the batch, adjust the factor only if the reference standards in the marker positions exceed the assigned value by more than $\pm$ 0.2 (see Note 4).

10.6.9 When all samples have been analyzed, a signal will sound, the analyzer will go into a 15 minute wash cycle and then enter the STANDBY mode. If the START button is not pressed within one hour to initiate another run, the power will automatically shut off.

10.6.10 To generate a printout of the results at the completion of the run, press the LIST button. Power must be on to generate printout, so this function may be done prior to the next day's run. Tape results printout on the back of the protocol sheet.

10.6.11 **Results**

10.6.12 Label each injection (chromatogram) on the printout tape and examine each chromatogram to make sure that the baseline is drawn correctly. The retention time of HbA1b should be 2.3 $\pm$ 0.1 minutes and that of HbA1c 4.4 $\pm$ 0.2 minutes. Record % HbF, HbA1c and HbA1 on the protocol sheet. When HbF does not appear in the progression of peaks and no HbF value is printed, report HbF as 0.0%. Be sure to note any abnormal peak and its position within the chromatogram (see Note 5).

10.6.13 Report results for ARIC patients as follows:

Report % HbAlc values to one decimal place. Do not report HbA1 results. If abnormal hemoglobin peak is observed, report HbF and HbAlc as "NA", use comment code AB and note "HbS seen" on the results printout.

10.7 **Notes**

10.7.1 Chromatograms demonstrating proper baseline construction exhibit optimum HbAO peak areas falling between 4000-6000 mv/sec. Dilution studies using varying reagent syringe spacers indicated that the 50% spacer could be used with autodilutor 70450 to optimize HbAO area while continuing to use the 40% spacer (as directed by BioRad) for autodilutor 70105. Use of the 50% spacer instead of the 40% spacer with autodilutor 70450 does not affect the final results.

10.7.2 The Diamat main power switch generally remains on, supplying power to the autosampler cooler, fans and the CPU. However, if the main power supply has been turned off, the following procedure must be used to restart the system:

a. Set the AUTO/MNL switch on control panel to MNL and set PUMP switch to OFF.
b. Switch transformer ON.
c. Turn main power lever ON (located on back of Diamat). The CPU switch on the back of the Diamat will light up. First both red and green lamps will be lit; after 2-3 minutes, press in the CPU switch so that only the green lamp is lit.
d. Press operation panel power button (ON). Troubleshooting experience with printer head and autosampler problems has shown that sometimes the only remedy is to shut the system down completely and then bring it back up again. To shut Diamat down, follow the above sequence in reverse order. After system is restarted, generate a parameter printout and recheck parameters. Re-enter current parameter changes before starting run.

10.7.3 Sample hemolysates are stable for up to 24 hours in the refrigerated autosampler compartment. If a power failure occurs, make fresh hemolysates and repeat run if more than one hour has elapsed. Alternately, the sample rack may be covered with parafilm and placed in the refrigerator until power is restored, if less than 24 hours elapses since hemolysates were made.

10.7.4 The URG (stat) button provides an extra sample injection during the run and may be used to run a repeat or a standard out of programmed sequence. The stat well is located at the lower left of the autosampler compartment. When the URG button is pressed, the Diamat will finish analysis of the current sample before proceeding to the stat well. The extra sample will always be numbered "sample 00" on the printout. The analyzer will then pick up where it left off sampling.

10.7.5 If an abnormal hemoglobin peak is present, the extra peak will be designated with a number placing it in the progression of Hb peaks from Ala through AO. Abnormal peaks exhibiting areas of only a few percent are thought to be the result of oxidation and are sometimes seen in improperly stored specimens. Note the presence of such peaks on worksheet. If a small degradation peak amounting to $\leq$ 0.5% is noted between the Alc and AO peaks report the Alc and comment: "Denatured hemoglobin = 0.3%"

10.7.6 Levels of fetal hemoglobin up to 5% do not affect test results because HbF is completely resolved. At levels greater than 5%, resolution of the HbAlc peak is affected due to incomplete resolution of the HbF peak, which results in a decrease in the apparent %HbAlc. Consult a supervisor for interpretation and reporting of resutls in patients with HbF levels > 5%.

10.8 References

1. Bio-Rad Diamat Automated Glycosylated Hemoglobin Analyzer Operation Manual, 1985. Cat. No. 196-1001. Bio-Rad Clinical Division, 1000 Alfred Nobel Drive, Hercules, CA 94547.

2. Bulletin 4250, Instruction Manual for Bio-Rad Diamat Hemoglobin Alc Reorder Pack, 1985. Cat. No. 196-1005. Bio-Rad Clinical Division.

3. Bulletin 4237, Instruction Manual for Bio-Rad Hemoglobin Alc by Column Test, 1983. Bio-Rad Clinical Division.

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11. CALIBRATION AND QUALITY CONTROL

11.1 Introduction

The University of Minnesota Hospital and Clinic (UMHC) has a long standing quality control program to minitor the performance of assays. ARIC specimens will be processed under this established program. Control material used by the UMHC chemistry laboratory will be used by the CCL for monitoring the corresponding procedures of the ARIC study. The following components are part of the UMHC on-going quality control system.

11.1.1 Reference Standards

Aqueous standards or protein-based calibrators are run with all batches wherever practicable. New stock standards are prepared at least once per year. Where applicable, NBS standard reference material is used for the standard or used to check the standard. Standards are checked to a tolerance of ± 1% of the nominal value before introduction into the procedure. New lots of calibrators are also checked and must meet certain criteria before being accepted.

11.1.2 Controls

In order to minimize the hepatitis risk, a commercial lyophilized or liquid bovine serum control is used for many procedures. Our own frozen serum pools prepared in the UMHC laboratory are used for some procedures. For all controls, our own means are established based primarily on calibration with our own aqueous reference standards. Controls are introduced into each run, under the premise that every unknown determination deserves a control. At least one control level in duplicate is included in every run.

11.1.3 Control Limits

Precision data is computed in two ways. Table 3 shows typical analytical data. Means will change depending on control material and precision data may change slightly. Initially, data from 100 runs of a procedure is used for both calculations. Within-day precision is computed using range statistics (R) and three standard deviation ($3SD_w$) limits are used. Overall precision is computed and two standard deviation ($2SD_o$) limits are used with this method. Both sets of limits are set around the mean of the procedure. The limit closer to the mean (whether it is $2SD_o$ or $3SD_w$) is used as a warning limit, and the limit further from the mean is used as an action limit. If the control is outside the action limits, results of unknowns cannot be sent out without remedial action, or consultation with the appropriate lab director.

11.1.4 Review of Control Values

All control values are plotted by hand or printed out by the DACOS on their respective charts. These are reviewed by the technologists in each division of the Chemistry Laboratory who initiate troubleshooting if indicated. If unable to solve the problem, discussion with the division director and/or laboratory co-director may lead to new suggestions for correcting the problem. Routinely, the charts are reviewed and initialed by the co-director monthly at a meeting with the laboratory managers and senior technologists. Troubleshooting is documented on the back of the control chart and reagent changes, etc., are kept in an auxiliary log with each chart.

11.2 Calibrators and Standard Solutions

11.2.1 DACOS Calibrators (I, II, III)

The three level set is supplied as lyophilized human serum pools with some added constituents. When reconstituted it simulates human serum and provides analyte concentrations suitable for calibrating the DACOS. This material is used to calibrate the following procedures: creatinine, glucose and uric acid.

11.2.2 Magnesium Standard

An aqueous standard is used for calibrating the magnesium procedure. The stock standard from the atomic absorption spectrophotometric method for magnesium is used. (See section 7.3.3 for details.)

11.2.3 Uric Acid Calibrators

DACOS calibrators (I, II, III) that have had values assigned to them by UMHC are used. The values have been obtained by correlation. (See section 11.3.2 for details.

11.2.4 DART NaK Standard Solution A and B

Aqueous standard solutions for the calibration of sodium and potassium.

11.2.5 Insulin Standards

Standards come as part of the Cambridge Kit. (See section 14.5.1.e for details.)

11.2.6 Hemoglobin Alc Standard.

Standard is included in the DIAMAT HbAlc Reorder Pack.
(See section 14.5.1 for details.)

11.3 Guidelines for Checking Calibrators on the DACOS

11.3.1 Sodium/Potassium Aqueous Calibrators

To check a new lot number of Standard A or Standard B:

1. Place the current lot number and the new lot number of the standard into sample cups on the sample tray in duplicate.

2. Request sodium and potassium assays to be run on the solutions.

3. Evaluate the results by comparing the average results of the new lot number with the average results of the old lot number.

$$\frac{\text{Average of new lot number}}{\text{Average of old lot number}} \times 100 = 99\text{-}101\%$$

4. If the results do not fall within the acceptable range, repeat the procedure again. Consult with lab manager if questionable results are obtained.

11.3.2 DACAL Calibrators

Each new lot number of DACAL calibrators contains an information sheet stating the calibrator concentration for all procedures (Calcium, Creatinine, Glucose). The assays listed are the original procedures as set up by Coulter. The stated calibrator concentration should be the correct value.

To evaluate the set of calibrators:

1. Run the three levels of the current lot number of DACAL's as samples to verify that the values are close to their set values in the test definitions.

2. Run the three levels of the new lot number of DACAL's in duplicate as samples.

3. Repeat procedure for three days.

4. Evaluate the results by comparing the average of these concentrations to Coulter's stated calibrator concentrations for each level of concentration for each of the assays.

$$\frac{\text{Avg. measured results (I + II + III)}}{\text{Avg. stated concentrations (I + II + III)}} \times 100 = 99\text{-}101\%$$

If the results fall within the acceptable range, use Coulter's stated concentration for calibrators in the test definition. If results do not fall within the acceptable range, other methods of evaluation can be used.

a. Evaluate the highest level calibration.

$$\frac{\text{Avg. measured value (III)}}{\text{Avg. stated concentration (III)}} \times 100 = 99\text{-}101\%$$

b. Graph both sets of values to see which set makes the "best" line.

c. Set up parallel runs by creating two test definitions for the assay using both sets of values. Run duplicate samples and compare to assay currently in use. Consult with lab manager if unclear results are obtained.

Uric Acid: The values on the information sheet received with the DACAL calibrators cannot be used. The uric acid assay is not a Coulter method, so values must be assigned to the calibrators. To initially determine values for the calibrator, a correlation study was done with another Chemistry Laboratory method that is standardized with primary aqueous standards. New lot numbers of calibrators are introduced as follows:

1. Run the current lot number of DACAL;s as samples to verify that the results agree with the set values in the test definitions in each assay.

2. Run the three levels of the new lot number DACAL's in duplicate as sample for five days.

3. Average the results of each level.

4. Create separate test definitions for each assay using these new values for the calibrators. The limits for slope, intercept, and calculated SD will have to be opened up wide until data for these limits can be obtained.

5. Run duplicate samples on this new test definition as well as the assay currently in use.

When the new lot number of DACAL calibrators are put into use, change the calibrator values in the test definitions for the assays. Check the assays with 5-10 duplicate specimens from main chemistry to verify correlation with the other methods. Monitor the quality control closely to detect any shifts due to the change. Consult with lab manager if any problems occur.

11.4 Guidelines for Checking Calibrator of Magnesium

See the Magnesium procedure (6.3.3).

11.5 Controls

Two levels of human-based serum are used for controls for the procedures on the DACOS. The insulin procedure is monitored with three lyophilized human based controls at various levels of insulin. Controls follow specimens through each procedure and are subjected to the same conditions as the donor specimens. Controls that are run in replicate serve as a source for statistical data for each analyte.

11.6 Establishing a Permanent Average Difference Between Duplicates (R), Standard Deviation Within Day (SD_w) and Standard Deviation Overall (SD_o)

11.6.1 Introduction

Calculations are made from 100 pairs over 100 days. Fewer pairs may be used on procedures that are not performed daily, but 50 pairs are minimal. Choose pairs randomly, i.e., at varied times during the batch. Be careful not to bias the results by eliminating poor duplicates (e.g., for 25 pairs, use the first two controls; for 25 pairs, use controls run in the middle of the day; for 25 pairs use pairs from the end of the day, etc.).

11.6.2 Calculate a mean and standard deviation overall (SD_o) using one control result from each day using a calculator with a program for SD.

11.6.3 Calculate R, which is the average difference between pairs.

11.6.4 Calculate one standard deviation within day (SD_w) from the following formula:

$$\frac{\bar{R}}{1.128}$$

11.6.5 Calculate duplicate ranges

a. Use $3.27 \times \bar{R}$
b. For methods with a wide range of values, use a percentage to determine duplicate range at a level much lower or higher than the control, e.g.,

$$\frac{SD}{\text{control mean}} \times 100 = \%\ \text{coefficient of variation (C.V.)}$$

Duplicate range should be: $\pm \frac{\%\ \text{C.V.} \times \text{level}}{100}$

11.7 Introducing a New Control

11.7.1 When a new control is put into use, a mean is calculated from 20 pairs run concurrently with the old control over 20 days. Fewer pairs may be used on procedures that are not performed daily, but 10 pairs are minimal, and any mean established from fewer than 20 pairs must be labelled "temporary" and reestablished when 20 pairs are available.

11.7.2 Calculate $\bar{R}$ from these pairs.

11.7.3 Enter the required information on the Cumulative Control Tabulation Sheet including the new R and the fact that the old R of x-value will continue to be used. The calculated R at this point serves to monitor the permanent R which was established from 100 pairs. It should not be used to make new ranges. If the R appears to be getting larger or smaller, new R, SD_w, and SD_o may be calculated from a new set of 100 pairs. A decision to change the permanent statistics on the basis of this data should be approved by the laboratory manager or laboratory director.

11.8 New Method or Instrumentation

11.8.1 If a new method or instrument is put into use, a temporary range should be used until 100 pairs can be accumulated.

11.8.2 The temporary range can be determined from calculations based on 10-20 pairs or using the mean from 10-20 pairs and R, SD_w, and SD_o calculated for the previous methodology.

11.9 Control Ranges Tabulation

Each laboratory section should keep this sheet current by writing on new controls. If changes have been made to this sheet, it should be updated and a copy of the new sheet given to appropriate faculty..

11.10 Control Charts and Ranges

11.10.1 Plot the mean, ± $2SD_o$ and ± $3SD_w$ ranges on permanent graph paper. From this, make xerox copies for the monthly charts. (Statistically, $2SD_o$ and $3SD_w$ ranges are similar and about half the time the $2SD_o$ range will be wider than $3SD_w$, and about half the time vice versa.)

11.10.2 Plot all controls daily. For those methods obtaining more than 10 controls daily, average the controls and plot the mean value, the highest value, and the lowest value. Also, record the number of controls used and the initials of the person doing the plotting. DACOS control charts are printed out at the end of the month.

11.10.3 Controls falling between the narrow and wide limits warn of trouble with the assay. Controls falling outside the wider limits require action be taken before results may be reported.

TABLE 3. Central Chemistry Laboratory Typical Analytical Precision[1]

Analyte	Units	Material[3]	Mean	Within Run[2] S.D.	Within Run[2] C.V.%	Overall S.D.	Overall C.V.%
Sodium	meq/L	LBS	117.5	0.93	0.8	0.83	0.7
		LBS	139.6	0.57	0.4	1.00	0.7
Potassium	meq/L	LBS	2.93	0.02	0.7	0.03	1.0
		LBS	4.55	0.04	0.9	0.05	1.5
Glucose	mg/dL	LBS	83.2	1.60	1.9	1.93	2.3
		LBS	134.3	3.07	2.3	3.16	2.4
Creatinine	mg/dL	LBS	1.07	0.05	4.7	0.04	3.7
		LBS	2.00	0.06	3.0	0.08	4.0
Magnesium	meq/L	LBS	2.33	0.12	5.1	0.13	5.6
		LBS	2.49	0.12	4.8	0.13	5.2
Uric Acid	mg/dL	LBS	3.02	0.16	5.3	0.22	7.3
		LBS	5.71	0.17	3.0	0.23	4.0
Insulin	μU/mL	LHS	8.0	1.01	12.8	2.60	32.5
		LHS	51.6	1.83	3.5	6.18	11.9
		LHS	116.8	3.26	2.8	11.39	9.8
Hemoglobin A1c	%	Frozen	5.30	0.15	2.8	0.18	3.2
			8.46	0.14	1.7	0.21	2.5

Data is based on fifty days run.

[1]Control chart warning and action limits are calculated as ± 3 SD within-run and ± 2 SD overall. Depending on the relative magnitude of the SDs, either the ± 3 SD within-run or the ± 2 SD overall may be the wider limits.

[2]Data based on range statistics using a set of duplicate control values within each run.

[3]Control material are liquid bovine serum (LBS) and lyophilized human serum (LHS).

APPENDIX I. Addresses and Phone Numbers

Collaborative Studies Coordinating Center
Department of Biostatistics
CB #8030, Suite 203, NCNB Plaza
The University of North Carolina at Chapel Hill
Chapel Hill, NC 27514-4145
Telephone: 919-962-6971

FIELD CENTERS

Forsyth County Center
Atherosclerosis Risk in Communities Study
2060 Beach Street
Winston-Salem, NC 27103
Telephone: 919-777-3040

Jackson Center
Atherosclerosis Risk in Communities Study
3000 Old Canton Road
Woodland Hills Building
Jackson, MS 39216
Telephone: 601-354-6149

Minneapolis Center
Atherosclerosis Risk in Communities Study
Suite 403 Oakdale Medical Office Building
3366 Oakdale Avenue North
Robbinsdale, MN 55422
Telephone: 612-627-4253

Washington County Center
Atherosclerosis Risk in Communities Study
5 Public Square
Professional Arts Building
Hagerstown, MD 21740
Telephone: 301-791-1847

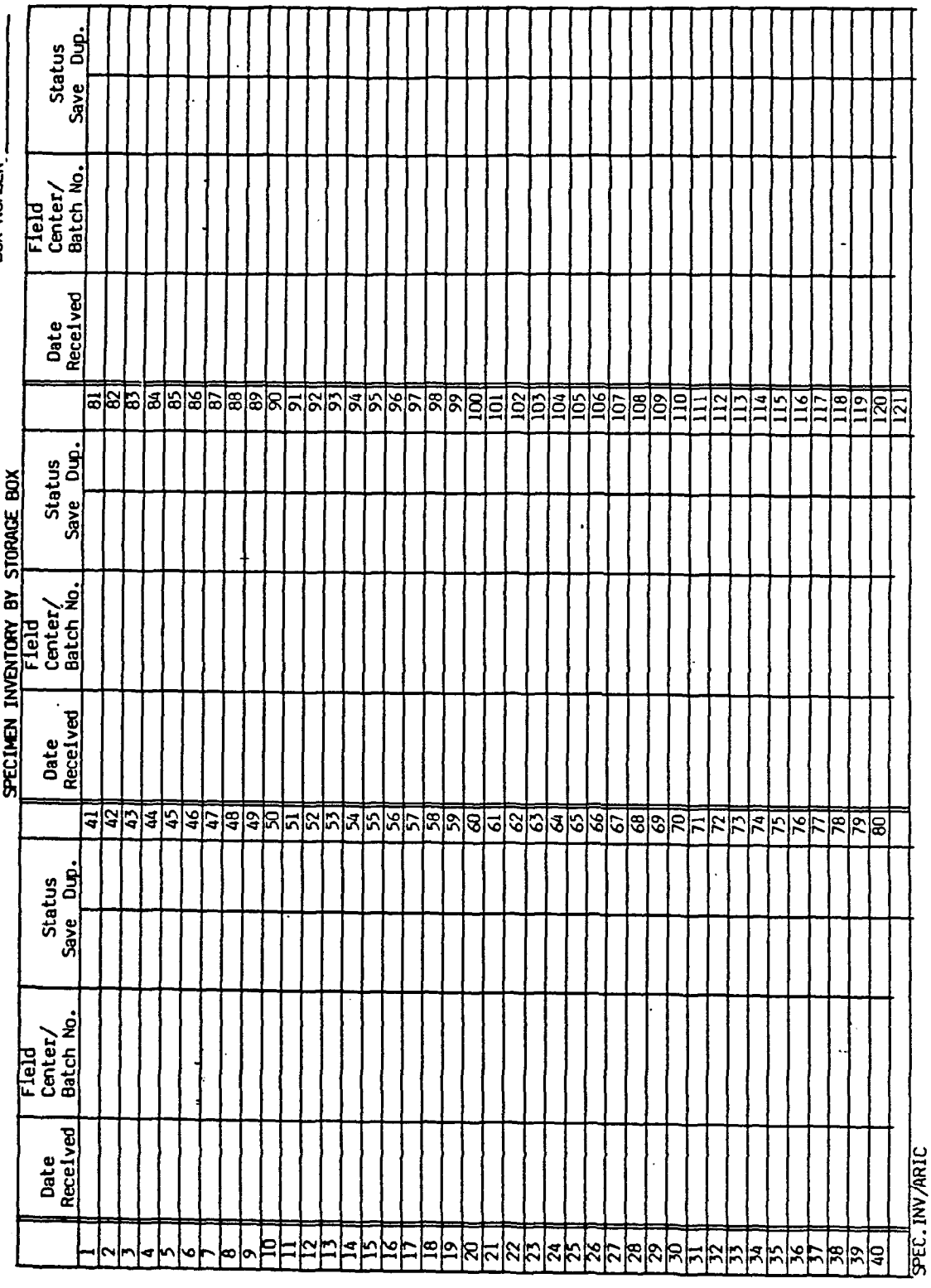
APPENDIX II

ARIC ALERT VALUES

DATE of RUN	BATCH NO.	PARTICIPANT ID NO.	TEST	ALERT RESULTS	NON-ALERT ABNORMAL RESULTS	DATE TRANS. REC'D	
						Data	Rec't

ARIC.AV/ARIC2 10-16-87

APPENDIX III



BATCH LOG

	Date Rec'd	Batch Number	No. of Spec	Location Box - Pos.	Sent Awkn.	Tests Run Insulin	Tests Run DACOS	Results In comp.	Results Ck'd	Results Disc No.	Date results mailed to field center	Misc.
1												
2												
3												
4												
5												
6												
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10												
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BATCH.LOG/ARIC1