Manual 8

Laboratory Methods

Visit 5

September 24, 2012 - Version 1

Study website - http://drupal.cscc.unc.edu/aric/

Laboratory Determinations
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1. BIOSPECIMEN COLLECTION AND PROCESSING

All specimens for the ARIC Visit 5 Study are collected at the four field sites. Preparation, collection handling and storage are in accordance to the guidelines of The Atherosclerosis Risk in Communities Study Manual # 7, Visit 5, "Blood and Urine Collection and Processing."

Informed consent forms must be obtained for each participant to ensure that the participants understand the purpose of blood drawing and the possible complications of venipuncture.

1.2. Blood

For blood samples that are sent to the ARIC Atherosclerosis (ACRL), the ARIC University of Minnesota (UMN) and the ARIC University of Texas Genetics (UTG) laboratories, strips of pre-numbered adhesive barcoded ARIC participant ID labels for each Vacutainer tube, each plastic micro sample storage tube, the specimen bags and packing list are attached to the initial data collection forms. Apply labels to the blood collection tubes for each participant 24 hours prior to blood collection. The ACRL will receive (49) 0.5 mL and (3) 1.0 mLfrozen plasma aliquots from (3) Na_2 EDTA powder tubes (tubes #4, #5 and #7).,Tube #6 will be treated with a cocktail, prepared at the ACRL Laboratory and shipped to the Field Centers, to prevent oxidation of the sample. Tubes #4, #5 and #7 will not be treated., Two 4.5 mL citrate tubes will be collected and (6) 1 mL aliquots made with 3 aliquots going to the ACRL and 3 aliquots to UMN. Additionally, (16) 0.5 mL frozen aliquots from (2) serum tubes will be collected. Aliquots of 500 µL will be stored in plastic vials with o-ring inserted screw caps. **Note: (1) 0.5 mL aliquot of whole blood collected before centrifugation** used for Hemoglobin A1c measurement.

The ARIC Field Centers will package all aliquots separating the ACRL and UMN aliquots into labeled bags.

See the Appendix 1 for a table of blood specimen collection, aliquoting and shipping.
See Appendix II for a diagram of packaging specimen aliquots to send to the ARIC ACRL.
See Appendix III for an illustration of packaging blood tubes (CPT, CBC and PAXgene) for Daily Shipment.

1.3. Urine Collection

Patients are provided with both written and spoken "clean-catch" instructions. When a clean-catch urine specimen is required, follow the directions given under Specimens Collection section of Manual #7. The collected urine should be refrigerated immediately or kept in a cool place to retard growth of bacteria until the test is performed.

2. LABORATORY METHODS: BAYLOR COLLEGE OF MEDICINE ATHEROSCLEROSIS CLINICAL RESEARCH LABORATORY (ACRL) – ARIC CENTRAL LABORATORY

2.1. Sample Processing And Storage At The Atherosclerosis Clinical Research Laboratory (ACRL)

Upon receipt of the weekly shipment from the field centers, the laboratory personnel will process samples using a large container filled with dry ice to assure thawing does not occur. The shipping form is stamped (date and time) and initialed. The bags and vials are inspected for status of samples (i.e. leakage, no label, etc.) and notes are made on the shipping form.
The field center(s) is notified if there is a problem with the shipment immediately.

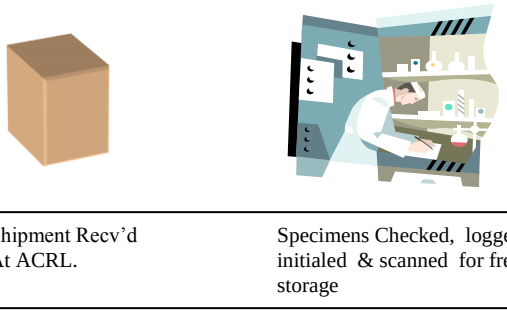
The labeled samples are checked against the shipping log for any discrepancies. If any are found the field center(s) is notified immediately. All email notifications are kept in a Public Folder accessible to all ARIC staff and retrievable. An Occurrence Folder with incident reports for error events, regardless of the source, is maintained.

The ACRL personnel will enter (using a barcode scanner) all sample I.D.'s into the freezer inventory database. The samples are placed into freezer boxes that hold 81 vials. Each box will be assigned a laboratory label as to its content. The boxes are then placed into freezer racks. The racks are placed into freezers. All samples are stored in -80º C freezers.

Diagram 1

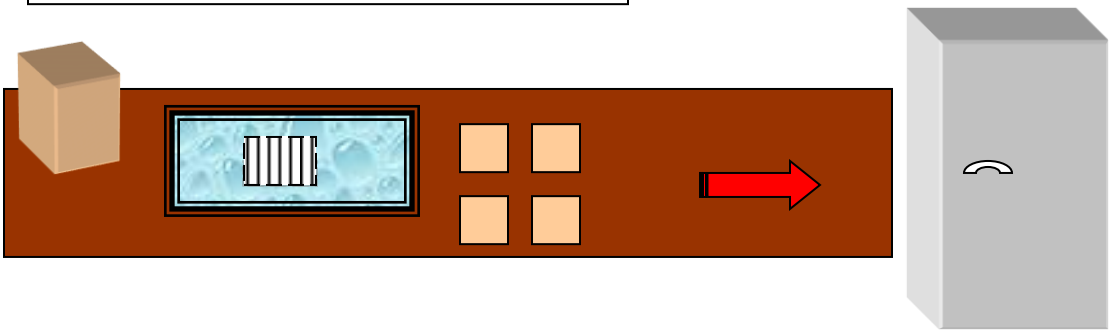
Flow Chart for Handling Frozen Specimens, Central Lipid Laboratory

(Universal Precautions Must Be Observed When Handling Bloodborne Pathogens)



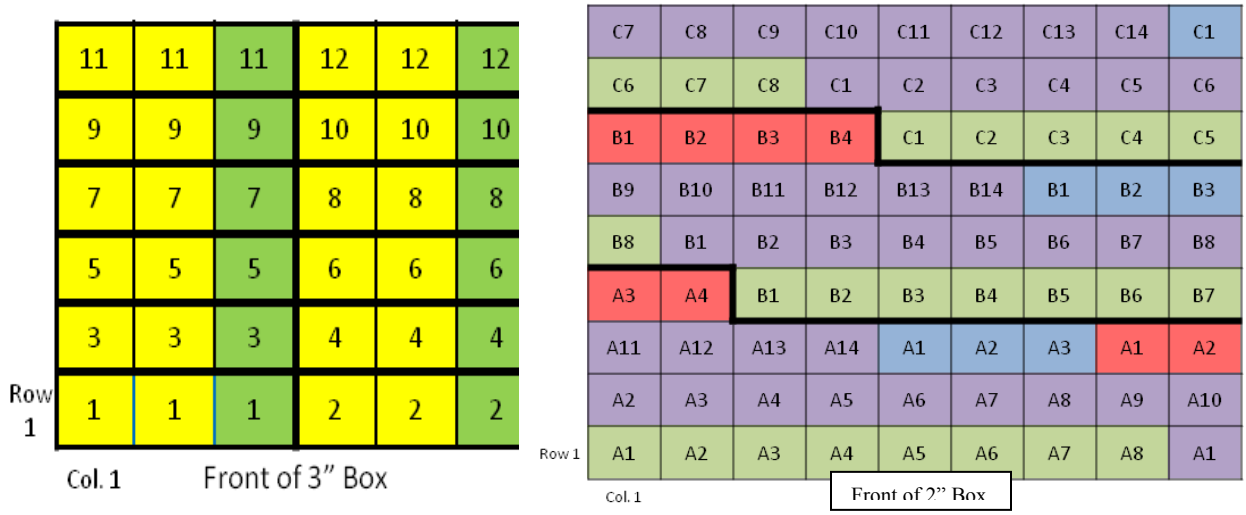
Shipment Recv'd At ACRL.

Specimens Checked, logged initialed & scanned for freezer storage



Samples— Processed on bed of dry ice Freezer boxes labeled Boxes are placed in labeled racks Stored in freezer

Diagram 2: Urine Aliquot Box Layout **Diagram 3: Blood Aliquots Box Layout**

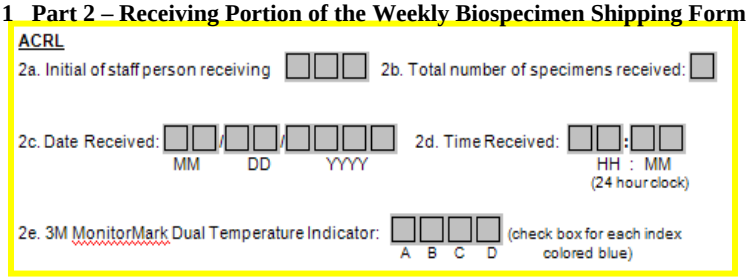


ACRL Weekly (Frozen)Processing Procedure

Part I. Sample Processing

A. Fill out **Part 2** of the **Weekly Biospecimen Shipping Forms** that are received with the package. (See Appendix IV for completed Weekly Biospecimen Shipping form Example)

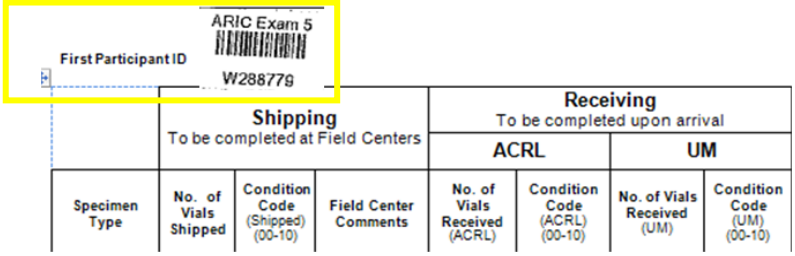
Fig. 1 Part 2 – Receiving Portion of the Weekly Biospecimen Shipping Form



B. Fill in the following data in the Microsoft Excel Visit 5 Database **for each participant before counting samples** (The Visit 5 Database is saved on the desktop of the computer at the front of the lab).

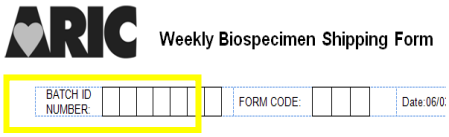
1. ARIC I.D. – scan the ARIC barcode label to prevent typing errors (found in Part 3 – Specimen Condition Log of the Weekly Biospecimen Shipping Form) (See Fig. 2)

Fig. 2 Participant ID barcode labels located in Part 3 of the Weekly Biospecimen Shipping Form



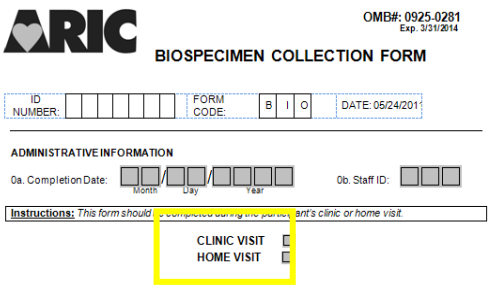
2. Batch ID # (found at the top of the Weekly Biospecimen Shipping Form). (See Fig. 3)

Fig. 3 Batch I.D.



3. Date received.
4. Initials of the lab technician(s) processing the samples.
5. Clinic or Home visit? This information is found on the first page of the Biospecimen Collection Form. (See Fig. 4)
6. Fasting? This information is found on page 2 of the Biospecimen Collection Form.

Fig. 4 Clinic or Home visit



For Sections C and D, see **Appendix IV**.

C. MN Sample Bags - complete steps 1-3 for each participant in the **same order** that they were added to the Visit 5 Database in Section B

1. Remove first participant's sample bag, check sample IDs on each vial to make sure they all match the ARIC I.D. scanned into the Visit 5 Database (do not remove vials from small bags).
2. Count the samples and record number of vials in the Visit 5 Database.
3. Place all participant bags from one batch in a large ziplock bag, and write the batch id on the bag.
4. Place the large MN bags on the top shelf of freezer #1 located in room F740.

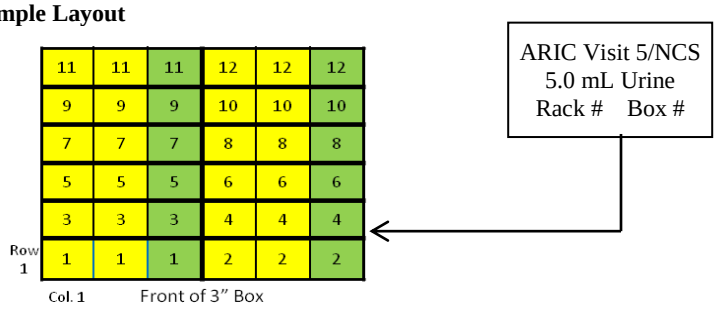
D. ACRL Sample bags - complete steps 1-3 for each participant in the **same order** that they were added to the Visit 5 Database in Section B.

1. Buffy Coats (brown cap)
   a. Remove the bags containing the Buffy Coat (brown cap) samples from each of the large participant sample bags and line them up in the same order as in section B.
   b. Check the sample IDs on each vial to make sure they all match the sample I.D. scanned into the Visit 5 Database (do not remove vials from bags).
   c. Record the number of vials in the Visit 5 Database.
   d. Gather the bags together (in the same order as section B) and secure with either a paper clip or rubber band.
   e. Place the bags in the small bin labeled "Buffy Coats Genetics Lab Weekly Pick-Up" on the top shelf of freezer #1 located in room F740.

2. Urine
   a. Remove the bags containing the urine samples from each of the large participant sample bags and line them up in the same order as in section B.
   b. Check the sample IDs on each vial to make sure they all match the sample I.D. scanned into the Visit 5 Database.

c. Record the number of vials in the Visit 5 Database.
d. Remove urine samples from bags (in the **SAME ORDER** as they are listed in the Visit 5 Database) and place in 3" box labeled "ARIC Visit 5/NCS, 5.0 mL Urine, Rack # Box#".
   i. Arrange each participant's samples by type. (see Fig. 5)
   (**leave a space for missing vials**)
e. Record rack and box # in the Visit 5 Database.

Fig. 5 Urine Box Sample Layout



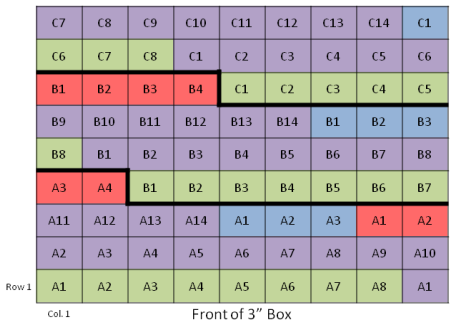
3. Remaining Samples
   a. Remove all the remaining small bags from the first participant's large sample bag.

*** The samples MUST be sorted into boxes in the SAME ORDER as they are listed in the Visit 5 Database to maintain an accurate inventory!**

b. Record the number of vials for each sample type in the Visit 5 Database.
c. Remove the green vials from their bag **first** and place in 2" box (See fig. 6) → ARIC Visit 5/NCS Freezer # Rack (AA, AB….) Box #
d. *Pull 1 EDTA (lavender) vial from its bag and place in 2" box labeled → ARIC Visit 5 0.5 mL EDTA Rack # Box #
e. Remove remaining EDTA (lavender) vials from their bag and place in box from step 3c (after the green vials), followed by the blue, and then red samples. (See Fig. 6) (**DO NOT leave spaces for missing vials**)
f. Record rack and box # in the Visit 5 Database.

* One EDTA vial is pulled from each participant for immediate testing. This aliquot is re-frozen for batch testing.

Fig. 6 Visit 5 Sample Layout



Part II. Documentation

A. Fill out Part 3 (Specimen Condition Log) of the Weekly Biospecimen Shipping Form

Fig. 7 Part 3 – Specimen Condition Log

	Shipping To be completed at Field Centers			**Receiving** To be completed upon arrival			
				ACRL		**UM**	
Specimen Type	**No. of Vials Shipped**	**Condition Code** (Shipped) (00-10)	**Field Center Comment**	**No. of Vials Received** (ACRL)	**Condition Code** (ACRL) (00-10)	**No. of Vials Received** (UM)	**Condition Code** (UM) (00-10)
Plasma **(Lavender)**	9	00		**9**	**00**		
Buffy Coat **(Brown)**	2	00		**2**	**00**	*** Genetics lab tech. initial here**	
Plasma **(Green)**	8	00		**8**	**00**		
Plasma **(Blue)**	6	00		**6**	**00**		
Serum **(Red/Gray)**	16	00		**16**	**00**		
A1c **(Black)**	1	00		**1**	**00**		
Urine **(Green)**	0	09		**0**	**09**		
Urine **(Yellow)**	3	00		**3**	**00**		

The "No. of Vials Received (ACRL)" column refers to each participant's **total number of vials per sample type**. Use the "Totals" section in the Visit 5 Database to accurately fill in the total number of vials received in the Specimen Condition Log of the Weekly Shipping Form.

1. Copy the **total** number of vials received for each participant from the Visit 5 Database to the ACRL Receiving section of the Weekly Biospecimen Shipping Form. (See Fig. 7)
2. Fill in the appropriate condition codes. (See Appendix III. for list of codes)
3. Make 2 copies of the Weekly Shipping and Receiving Forms included in the shipment (rev. # 1)
    a. Place the **original** Weekly Shipping and Receiving Forms in the binder labeled "ARIC Visit 5/NCS Study Weekly Shipping Forms" and the Biospecimen Collection Forms in one of the four binders (one for each field center) labeled "ARIC Visit 5/NCS Study Biospecimen Collection Forms".

b. One copy of the shipping forms will be included in the weekly shipment to MN, and the other copy will be given to the Genetics Lab when they pick up the Buffy Coats.

Part III. Shipping

A. Buffy Coats

1. A lab tech from the University of Texas-Houston Genetics Lab will pick up the Buffy Coat samples every Wednesday morning around 10:00 am.
    a. The genetics lab tech must **sign and date the original copy** of Part 3 of the Weekly Biospecimen Shipping Form **for every participant** located in the binder labeled "ARIC Visit 5/NCS Study Weekly Shipping Forms." (see fig. 7)
        i. It is the responsibility of the ACRL lab tech responsible for sample processing to ensure that all paperwork is completed by the Genetics Lab tech.
2. Update the "Date Picked Up" column in the Excel Visit 5 Database.

B. UMN Samples

Samples received in the bags labeled "MN" are generally shipped either the same day of receipt or the following day (no shipments are made on Thursday or Friday) to the University of Minnesota Advanced Research & Diagnostics Lab at the following address:

Deanna Gabrielson - Rm L275 Mayo Bldg.
Univ. of Minnesota - Biochem Lab.
420 Delaware Street S.E.
Minneapolis, MN 55455

1. Using a Biomailer shipping container, add a layer of crushed dry ice to the bottom of the Styrofoam box, followed by all the bags labeled MN, then another layer of crushed dry ice (Use 5-10 lbs. of dry ice).
    a. Be sure to check off each participant sample bag added to the biomailer to ensure no samples are skipped.
    b. Use more than one Biomailer if necessary, do not try to over pack the box.
2. Place packing material (do not use "packing peanuts") on top of the dry ice to fill the box.[1]
3. Place the Biospecimen Form and the **Weekly Shipping and Receiving Forms** in a zip lock and tape to the top of the Styrofoam lid.
4. Seal the box tightly with strapping tape. Affix a dry ice Class 9 label, UN3373 Biological Substance Category B label, contents description label and completed Federal Express air bill to the outside of the box. Select FedEx Priority Overnight for delivery by 10:30 am the following day.[1]
5. Contact Federal Express (1-800-GO-FEDEX) for pickup, and place the biomailer(s) on the counter in the 6th floor mail room.
6. Send an email to **dgabrie2@Fairview.org** with the FedEx tracking number(s).

C. Return Biomailers

1. The Biomailers received weekly from the field centers must be returned to them the same day if possible.

a. Include all packing materials (bubble wrap, bags, laminated MN and ACRL tags) belonging to that field center.
b. Mark out all biohazard and dry ice labels on the outside of the box.
c. Place the "To:" address label in the center of the box and the "From:" address label at the top left.

2. The Biomailers received by the UMN Laboratory are returned the next week to the ACRL.
3. The ACRL will occassionaly have to destroy biomailers that are not suitable for shipping. The field center is notified when this occurs and must manage their supply inventory.

ACRL Daily Sample Processing Procedure:

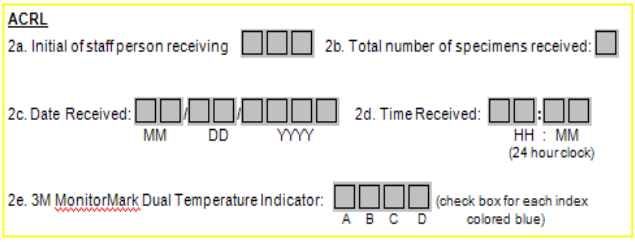
ARIC Visit 5/NCS

Daily Sample Processing Protocol for the ACRL lab

Step 1. Documentation

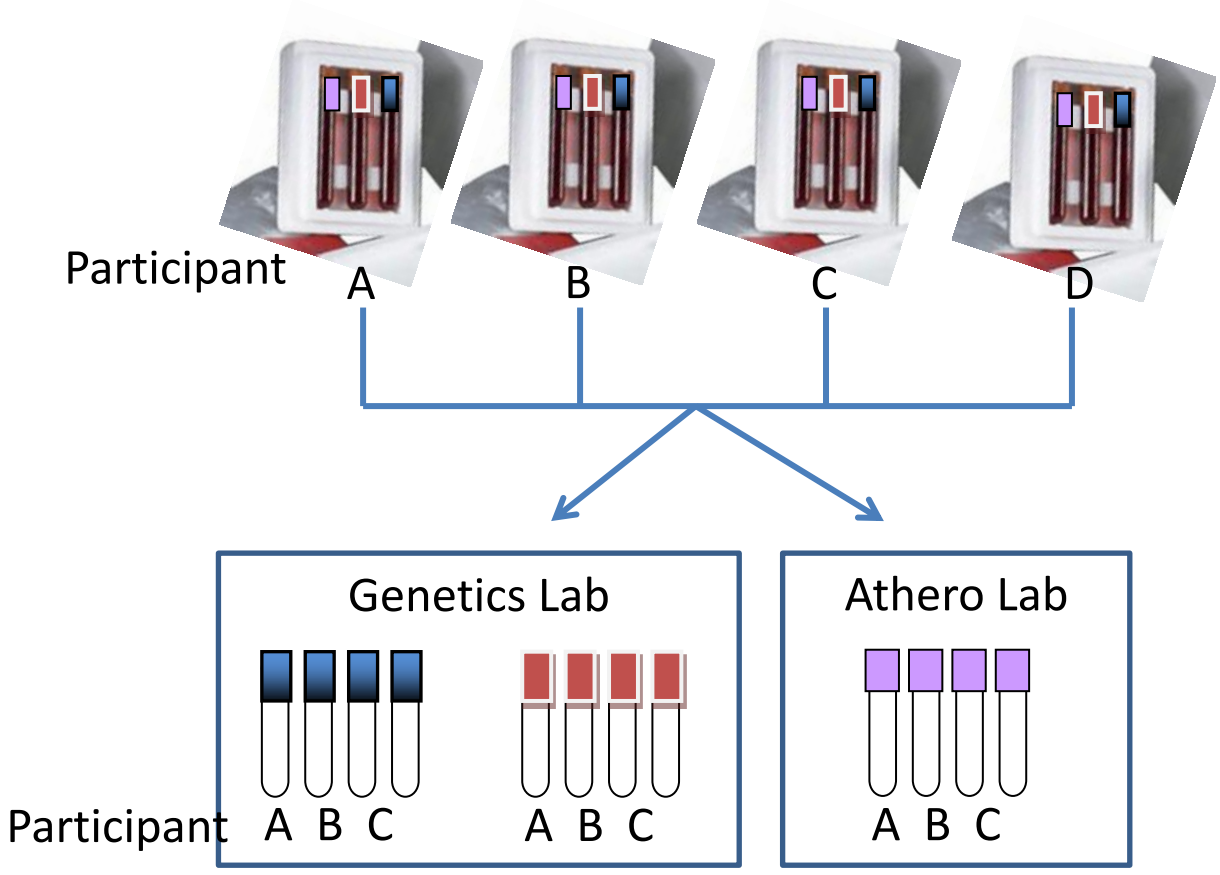
a. Fill out Part 2 – Receiving Log **AND** Part 3 – Specimen
Condition Log in the **Daily Shipping Form**

b. Update the Visit 5 database
(enter ARIC IDs into the database by scanning the ARIC



Step 2. Separate vials

Place CBC tubes (lavender caps) in one rack and the CPT and PAX tubes in another for the ARIC UT Genetics lab to pick up



Step 3. Run CBCs

Step 4. Submit Data

2.2. The Aric Atherosclerosis Clinical Research Laboratory (ACRL) Test Methods

2.2.1 Cholesterol

Principle:

Measurements of cholesterol are used primarily in the diagnosis and treatment of disorders involving excess cholesterol in the blood, and lipid and lipoprotein metabolism disorders.

Total serum/plasma cholesterol analysis has proven useful in the diagnosis of hyperlipoproteinemia, atherosclerosis, hepatic and thyroid diseases.[1] Total and HDL cholesterols, in conjunction with a triglyceride determination, provide valuable information for the prediction of coronary heart disease.[2]

Intended Use:

System reagent for the quantitative determination of cholesterol concentrations in human serum or plasma on OLYMPUS analyzers.

Methodology:

Assaying total cholesterol in saponified serum extracts using "cholesterol dehydrogenase" began with Flegg[3] and Richmond[4]. Previously, Hernandez and Chaikoff [5] and Hyun et al.[6] had isolated a cholesterol ester hydrolase which was effective in producing free cholesterol from cholesterol esters. Finally, in 1974, Allain et al.[7] and Rieschlau et al.[8] were able to combine the esterase and oxidase into a single enzymatic reagent for the determination of total cholesterol; this is the basis for the Olympus Cholesterol method.

The Olympus Cholesterol reagent has been certified to meet the National Cholesterol Education Program's (NCEP) performance criteria for accuracy.

Cholesterol esters in serum are hydrolyzed by cholesterol esterase (CHE). The free cholesterol produced is oxidized by cholesterol oxidase (CHO) to cholest-4-en-3-one with the simultaneous production of hydrogen peroxide (H_20_2), which oxidatively couples with 4-aminoantipyrine and phenol in the presence of peroxidase to yield a chromophore.

The red quinoneimine dye formed can be measured spectrophotometrically at 540/600 nm as an increase in absorbance.

Cholesterol esters $\longrightarrow$ **Cholesterol + Fatty acids**

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHO}} \text{Cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{Red dye} + 4H_2O_2$$

Controls and Standards

All standards and controls are tested in duplicate for quality control purposes. Various suppliers provide the controls that are used. Normal and abnormal internal control pools and calibrators are obtained from

Beckman Coulter Olympus. Additional control materials used for all laboratory measurements are obtained from U.S. Biologicals.

Specimen:

Patient Preparation:

None is required if only cholesterol is being measured. A 12-hour fast or greater is required if an entire lipid panel is being performed.

Type:

Serum, EDTA plasma, or heparinized plasma, free from hemolysis, are the recommended specimens. Separate serum from blood cells as soon as possible. Use of the following anticoagulants is not recommended: oxalate, citrate or fluoride.[10]

Total Cholesterol levels in EDTA plasma should be corrected by multiplying the result obtained by 1.03 to be equivalent to serum levels of Total Cholesterol.[9]

Handling Conditions:

Total cholesterol in serum is stable for 7 days when stored at 2-8°C, 3 months when stored ≤-20°C, and for over one year stored at -70°C[10].

Performance Parameters:

The following data was obtained using the Olympus System Cholesterol Reagent on an Olympus AU400®/AU400e®, AU600®, AU640®/AU640e®, AU680®, AU2700®, and AU5400® analyzers according to established procedures.

Precision:

Estimates of precision, based on CLSI recommendations[15], are consistent with typical performance. The within run and total precision is less than 3%. Assays of control sera were performed and this data reduced following CLSI guidelines.

Serum:					
AU400®/AU400e® Results	**N=60**	**Within run**		**Total**	
	Mean, mg/dL	**SD**	**CV%**	**SD**	**CV%**
	104.9	0.66	0.63	1.13	1.08
	247.4	1.98	0.80	3.36	1.36
	447.4	3.34	0.75	4.78	1.07

Reporting Results:

Reference Ranges:

Total Cholesterol	Risk Classification[13]
< 200 mg/dL	Desirable
200 - 239 mg/dL	Borderline High
> 240 mg/dL	High
Olympus Recovered Reference Range[14]	136 - 290 mg/dL

Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Procedures for Abnormal Results:

Abnormal results are flagged by the listed analyzers according to the normal values entered by the user into the instrument parameters.

Reporting Format:

Results are automatically printed for each sample in mg/dL at 37^{o} C.

Limitations:

The Olympus Cholesterol procedure is linear from 25 to 700 mg/dL. Samples exceeding the upper limit of linearity should be diluted and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically utilizing the AUTO REPEAT RUN.

Interfering Substances:

Results of studies conducted[11] on the AU400®/AU400e®, AU600®, AU640®/AU640e®, AU680®, AU2700®, and AU5400® show that the following substances interfere with this cholesterol assay.

For AU400®/AU400e®

Ascorbate:	Interference less than 10% up to 10 mg/dL Ascorbate
Bilirubin:	Interference less than 10% up to 12 mg/dL Bilirubin
Hemolysis:	Interference less than 10% up to 500 mg/dL Hemolysate
Lipemia:	Interference less than 2% up to 1000 mg/dL Intralipid*

* Intralipid, manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

The information presented is based on results from Olympus studies and is current at the date of publication. Olympus America Inc., makes no representation about the completeness or accuracy of results generated by future studies. For further information on interfering substances, refer to Young[12] for a compilation of reported interferences with this test.

References:

1. Tietz, N.W., Fundamentals of Clincial Chemistry, Third Edition, WB Saunders, 448: 1987.
2. Gordon, T., Castelli, W.P., Hjortland, M.C., Kannel, W.B. and Dawber, T.R., Am J Med, 62: 707: 1977.
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8. Rieschlau, P., Bernt, E. and Gruber, W.,Z Klin Chem Klin Biochem, 12: 403: 1974.
9. Olympus America Inc., data on file.
10. Tietz, N.W.(ed), Clinical Guide to Laboratory Tests, Second Edition, W.B. Saunders, 1990.
11. CLSI, Interference Testing in Clinical Chemistry, EP7-P, 1986.
12. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, Fifth Edition, AACC Press, 2000.
13. Report from the Laboratory Standardization Panel of the National Cholesterol Education Program, Clin Chem, 34: 193, 1988.
14. Olympus America Inc., data on samples collected from 200 blood donors in North Texas.
15. CLSI Evaluation Protocol, EP5-T2, 1992.

2.2.2. LDL-Cholesterol

LDL-Cholesterol is calculated using Friedewald's formula. The formula hinges on the assumption that VLDL-C is present in a concentration equal to one fifth of the triglyceride concentration. This assumption is valid for triglyceride concentrations less than 400 mg/dL: thereafter, inconsistencies in the VLDL triglyceride/cholesterol ration occur, and the formula cannot be used. Thus, by measuring total cholesterol, triglyceride, and HDL-C levels, the LDL-C level may be calculated as LDL-C=TC - (HDL-C) - TG/5.[8]

2.2.3 Triglycerides

Principle:

.Triglycerides are the major form of fat found in nature and their primary function is to provide energy for the cell.[1] Measurements of triglyceride are used in the diagnosis and treatment of patients with diabetes mellitus, nephrosis, liver obstruction, other diseases involving lipid metabolism, or various endocrine disorders.[2]

Clinically, triglyceride assays are used to help classify the various genetic and metabolic lipoprotein disorders, and in the assessment of risk factors for atherosclerosis and coronary artery disease.[3,4]

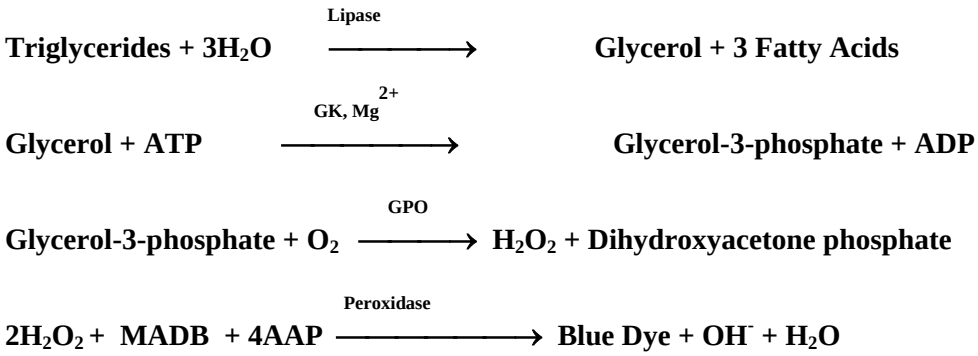
Intended Use:

System reagent for the quantitative determination of Triglyceride concentrations in human serum and plasma on OLYMPUS analyzers.

Triglycerides reagent OSR66118 is for use on the AU2700® and AU5400® analyzers only.

Methodology:

This Olympus Triglyceride procedure is based on a series of coupled enzymatic reactions.[5,6] The triglycerides in the sample are hydrolyzed by a combination of microbial lipases to give glycerol and fatty acids. The glycerol is phosphorylated by adenosine triphosphate (ATP) in the presence of glycerol kinase (GK) to produce glycerol-3-phosphate. The glycerol-3-phosphate is oxidized by molecular oxygen in the presence of GPO (glycerol phosphate oxidase) to produce hydrogen peroxide (H_2O_2) and dihydroxyacetone phosphate. The formed H_2O_2 reacts with 4-aminophenazone and N,N-bis(4-sulfobutyl)-3,5-dimethylaniline, disodium salt(MADB) in the presence of peroxidase (POD) to produce a chromophore, which is read at 660/800 nm. The increase in absorbance at 660/800 nm is proportional to the triglyceride content of the sample.



Specimen:
Patient Preparation:

The patent should be fasting, minimum water intake allowed, for >12 hours prior to sampling. Ideally, the patient should be on a stable diet for 3 weeks preceding the test and should not consume alcohol within 72 hours prior to the sampling. [15]

Type:

Fasting (≥12 hours) serum samples[7], free from hemolysis and removed from the clot are the recommended specimens. EDTA and heparin are the suggested anticoagulants if plasma must be used.

Ensure that all equipment used in the collection and storage of samples are free from glycerol contamination.

Handling Conditions:

Serum triglyceride is stable for seven days when stored at 2-8°C and 3 months when stored frozen at ≤-20°C.[8]

Equipment And Materials:
Equipment:

Beckman Coulter Olympus AU400e® analyzer.

Materials:

Olympus System Triglyceride Reagent

Final concentration of reactive ingredients:

PIPES buffer (pH 7.5)	50	mmol/L	
Lipase (Pseudomonas)	≥ 1.5	kU/L	(25 μkat/L)
Glycerol kinase (Bacillus stearothermophilus)	≥ 0.5	kU/L	(8.3 μkat/L)
Glycerol phosphate oxidase (Pseudomonas)	≥ 1.5	kU/L	(25 μkat/L)
Ascorbate oxidase (Curcubita species)	≥ 1.5	kU/L	(25 μkat/L)
Peroxidase (Horseradish)	≥ 0.98	kU/L	(16.3 μkat/L)
ATP	1.4	mmol/L	
4-Aminoantipyrine	0.50	mmol/L	
Magnesium acetate	4.6	mmol/L	

Preparation:

The Olympus System Triglyceride Reagent is liquid, ready for use. No preparation is needed.

For OSR66118, insert the pipe supplied into the 180mL Triglycerides reagent bottles before use on the analyzer. Care must be taken when handling the pipe to avoid contamination. The pipe is for single use only. Do not remove the large cap.

The Olympus Chemistry Calibrator reconstitution:

- Remove the vials of calibrator and diluent from storage and let stand at room temperature (18-28°C) for 5 minutes.
- Remove the cap and stopper from the vials of the lyophilized serum and reconstituting diluent.
- Using a volumetric pipette or a calibrated air-displacement pipettor, add exactly 5.0 mL of reconstituting diluent to DR0070 lyophilized serum vial. DO NOT pour directly from the reconstituting diluent vial.
- Replace the cap and stopper to the vial of lyophilized serum immediately after adding the diluent

- Allow the calibrator to stand for 5-10 minutes. Gently swirl the contents until completely dissolved.

Storage Requirements:

1. The unopened reagents are stable until the expiration date printed on the label when stored at 2 - 8°C.
2. Opened reagents are stable for 30 days when stored in the refrigerated compartment of the analyzer.
3. Unreconstituted calibrator and diluent are stable until the expiration date stated on the label when stored at 2 - 8°C.
4. For Triglycerides, reconstituted calibrator materials are stable for 7 days from the date of reconstitution when stored at 2 - 8°C. The materials should be capped and stored upright 2 - 8°C when not in use

Indications of Deterioration:

Discoloration of the reagent, visible signs of microbial growth, gross turbidity or precipitation in reagent may indicate degradation and warrant discontinuance of use.

Performance Parameters:

The following data was obtained using the Olympus System Triglyceride Reagent on an Olympus AU400®/AU400e®, AU600®, AU640®/AU640e®, AU680®, AU2700®, and AU5400® analyzers according to established procedures.

Precision:

Estimates of precision, based on CLSI recommendations[14], are consistent with typical performance. The within run precision is less than 3% and total precision is less than 5%. Assays of serum pools were performed and the data reduced following CLSI guidelines.

Serum:					
AU400®/AU400e® Results	**N=80**	**Within run**		**Total**	
	Mean, mg/dL	**SD**	**CV%**	**SD**	**CV%**
	88.9	1.09	1.22	2.30	2.58
	190	1.72	0.90	4.84	2.54
	439	4.64	1.06	10.60	2.41
Serum:					
AU600® / AU640® / AU640e® / AU680® Results	**N=80**	**Within run**		**Total**	

	Mean, mg/dL	SD	CV%	SD	CV%
	89.4	0.57	0.64	1.48	1.65
	191	0.95	0.49	2.69	1.41
	442	2.28	0.51	6.45	1.46
Serum:					
AU2700®/AU5400® Results	**N=80**	**Within run**		**Total**	
	Mean, mg/dL	**SD**	**CV%**	**SD**	**CV%**
	90.3	0.54	0.60	1.81	2.00
	192	0.85	0.44	3.30	1.72
	443	3.11	0.70	7.90	1.78

Comparison:
Serum:

Patient samples were used to compare this Olympus System Triglyceride Reagent on the AU640®/AU640e® to another Olympus analyzer Triglyceride method (Method 2). Further studies were conducted as outlined below.

Y Method	**AU600®/AU640®/AU 640e®**	**AU400®/AU400e®**	**AU2700®/AU5400®/ AU680®**
X Method	**Method 2**	**AU640®/AU640e®**	**AU640®/AU640e®**
Slope	1.011	1.023	0.970
Intercept	-0.871	-2.232	2.783
Correlation Coeff. (r)	1.000	1.000	1.000
No. of Samples (n)	148	148	148
Range (mg/dL)	14 - 939	13 - 953	13 – 953

Sensitivity:

The lowest detectable level using serum settings was estimated as follows:

Analyzer	Lowest Detectable Level (mg/dL)
AU400®/AU400e®	0.31
AU600®/AU640®/AU640e®/AU680®	0.31
AU2700®/AU5400®	0.26

The lowest detectable level represents the lowest measurable level of Triglyceride that can be distinguished from zero. It is calculated as the absolute mean plus three standard deviations of 20 replicates of an analyte free sample.

Functional Sensitivity

Serum:

Precision results (40-fold determination) for a level < 5.0 mg/dL are shown below with a CV of < 20% for each application.

Analyzer	Mean Conc. (mg/dL)	SD	CV
AU400®/AU400e®	3.47	0.310	8.9
AU600®/AU640®/AU640e®/AU680®	4.07	0.560	13.7
AU2700®/AU5400®	4.09	0.478	11.7

Calibration:
Standard Preparation:

Perform a one-point calibration (AB) using a water blank (blue rack) and the appropriate calibrator in a yellow calibration rack. The frequency of calibration is every 30 days. Calibration of this triglyceride procedure is accomplished by use of Olympus Calibrator material, which is traceable to the College of American Pathology (CAP) Serum Lipid (RM016)#2.

Calibration Procedure:

Recalibration is required when any of the following conditions occur:

1. A reagent lot number has changed or there is an observed shift in control values.
2. Major preventative maintenance was performed on the analyzer.

3. A critical part was replaced.

Quality Control:

During operation of the Olympus analyzer at least two levels of an appropriate control material should be tested a minimum of once a day. In addition, controls should be performed after calibration with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate Olympus User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Analyzer Parameters:

A complete list of test parameters and operating procedures can be found in the appropriate User's Guide.

Calculations:

For SI Units (mmol/L), multiply the results by 0.0113.

REPORTING RESULTS:

Reference Ranges:

Triglyceride Level	**Risk Classification[12]**
< 150 mg/dL	Normal
150-199 mg/dL	Borderline High
200-499 mg/dL	High
≥ 500 mg/dL	Very HIgh

General population range as recovered by Olympus America Inc.: Adults[13]: 48 - 352 mg/dL

Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Procedures for Abnormal Results:

Abnormal results are flagged by the listed analyzers according to the normal values entered by the user into the instrument parameters.

Reporting Format:

Results are automatically printed out for each sample in mg/dL at 37°C.

Limitations:

This Olympus Triglyceride procedure is linear from 10 to 1000 mg/dL. Samples exceeding the upper limit of linearity should be diluted and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically utilizing the AUTO REPEAT RUN.

Note: Triglycerides GPO enzymatic methodologies are subject to a strong negative interference from patient samples with extremely elevated triglyceride levels[11]. While these samples are extremely lipemic in appearance and have triglyceride levels exceeding 1700 mg/dL, results can be erroneously reported as being within the linear range of the assay. In order to identify grossly lipemic samples exhibiting this phenomenon, Data Check Parameters have been provided. If the reaction kinetics of a test exhibits the characteristics of one of these elevated triglyceride samples, the analysis result will be flagged ("F", "Z", @, or &). Under rare circumstances, grossly lipemic samples may evade the Data Check Parameters and should be diluted 1 part sample to 4 parts saline prior to analysis and the results multiplied by 5.

Interfering Substances:

Results of studies[9] conducted on the AU400®/AU400e®, AU600®, AU640®/AU640e®, AU680®, AU2700®, and AU5400® analyzers show that the following substances interfere with this triglyceride procedure.

For AU400®/AU400e®

Ascorbate:	Interference less than 5% up to 20 mg/dL Ascorbate
Bilirubin:	Interference less than 3% up to 40 mg/dL Bilirubin
Hemolysis:	Interference less than 3% up to 500 mg/dL Hemolysate

The information presented is based on results from Olympus studies and is current at the date of publication. Olympus America Inc., makes no representation about the completeness or accuracy of results generated by future studies. For further information on interfering substances, refer to Young[10] for a compilation of reported interferences with this test.

References:

1. Kaplan, L.A. and Pesce, A.J. (eds), Clinical Chemistry Theory, Analysis and Correlation, Third Edition, C.V. Mosby Co., 465, 1996.
2. Davidson, I. and Henry, J.B., Clinical Diagnosis by Laboratory Methods, Fifteenth Edition, WB Saunders, 624, 1974.
3. Gordon, T., Castelli, W.P., Hjortland, M.C., Kannel, W.B. and Dawber, T.R., Am J Med, 62: 707, 1977.
4. Fredrickson, D.S., et al., New Eng J Med, 276: 32, 1976.
5. Trinder, P., Ann Clin Biochem, 6: 24, 1969.
6. Bucolo, G. and David, H., Clin Chem, 19: 476, 1973.
7. Tietz, N.W., Clinical Guide to Laboratory Tests, Fourth Edition, WB Saunders, 2006.
8. Tietz, N.W., Textbook of Clinical Chemistry, WB Saunders, 888, 1986.
9. CLSI, Interference Testing in Clinical Chemistry, EP7-A, 2002.
10. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, Fifth Edition, AACC Press 2000.
11. Shephard, M.D.S. and Whiting, M.J., Clin Chem 36/2, 1990.
12. National Cholesterol Education Program (NCEP), Adult Treatment Panel, ATP III Guidelines, 2004.

13. Olympus America Inc. data on samples collected from 200 blood donors in North Texas.
14. CLSI Evaluation Protocol EP5-A, 1999.
15. Bennett, B.D., et al. (eds.), Patient Preparation and Specimen Handling Fascicle VI, College of American Pathologists, 1992.

2.2.4 HDL-Cholesterol

Principle:

Many epidemiological investigations have demonstrated the strong and independent inverse association between HDL-Cholesterol and the risk of coronary artery disease[1,2]. It has been proposed that HDL particles, through the uptake and transport of cholesterol from peripheral tissue to the liver (reverse cholesterol transport), protects against the development of atheromatous plaques[3].

Under the guidelines issued by The National Cholesterol Education Program Adult Treatment Panel 2 (NCEP ATP 2)[4], it is recommended that both HDL-Cholesterol and Total Cholesterol should be measured in the initial screening for hypercholesterolemia. In 2001, the NCEP increased the high-risk medical decision point to <40 mg/dL[5].

The guidelines classify HDL- C levels as follows:

1. < 40 mg/dL as indicative of a major risk factor for Coronary Heart Disease.
2. > 60 mg/dL as a negative risk factor for Coronary Heart Disease.

Intended Use:

System reagent for the quantitative determination of HDL-Cholesterol concentrations in human serum or plasma on OLYMPUS analyzers.

Methodology:

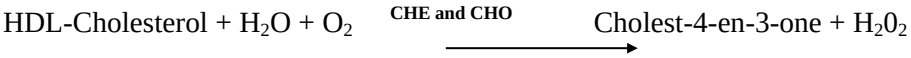
The Olympus HDL-Cholesterol test (HDL-C) is a two reagent homogenous system for the selective measurement of serum or plasma HDL-Cholesterol in the presence of other lipoprotein particles. The assay is comprised of two distinct phases. In phase one, free cholesterol in non-HDL-lipoproteins is solubilized and consumed by cholesterol oxidase, peroxidase, and DSBmT to generate a colorless end product. In phase two, a unique detergent selectively solubilizes HDL-lipoproteins. The HDL cholesterol is released for reaction with cholesterol esterase, cholesterol oxidase, and a chromogen system to yield a blue color complex, which can be measured bichromatically at 600/700nm. The resulting increase in absorbance is directly proportional to the HDL-C concentration in the sample.

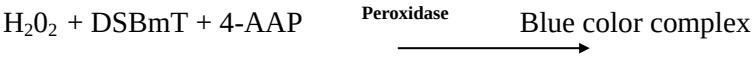
Reaction Phase 1

LDL, VLDL, Chylomicrons —(**Accelerator + CO**; **DSBmT + Peroxidase**)→ Colorless end product

Reaction Phase 2

HDL Cholesterol —(**HDL Specific detergent**)→ HDL Disrupted





This reagent was tested in a Cholesterol Reference Method Laboratory Network (CRMLN) laboratory to confirm that it meets the guidelines of the NCEP.

SPECIMEN:
Patient Preparation:

A twelve to fourteen-hour fast is recommended, but not required.[11]

Additional instructions for patient preparation as designated by this laboratory:

Type:

Serum, EDTA plasma, or heparinized plasma free from hemolysis is the recommended specimen. Separate serum from red blood cells as soon as possible (within 3 hours). Plasma using anticoagulants such as oxalate or citrate is not recommended.[6]

Handling Conditions:

Use fresh sample for analysis when possible. Serum or plasma should not remain at room temperature (15-25°C) longer than 14 hours. If analysis is not completed within 14 hours, serum or plasma may be stored refrigerated at 2-8°C for up to one week. If specimens need to be stored for more than 1 week, they may be preserved frozen at ≤-70°C for up to 3 months. Avoid repeated freezing and thawing of the sample. Samples should only be frozen once.[6]

Equipment And Materials:

Equipment:

- Beckman Coulter Olympus AU400®, analyzer.

Materials:

Olympus System HDL-Cholesterol Reagent

Final concentration of reactive ingredients:

Good's Buffer (pH 6.0)	
Cholesterol esterase (Pseudomonas)	375 U/L
Cholesterol oxidase (E. coli)	750 U/L
Peroxidase (Horseradish)	975 U/L
Ascorbate oxidase (Curcubita sp.)	2250 U/L
DSBmT	0.75 mmol/L
4-aminoantipyrine	0.25 mmol/L
Detergent	0.375%

Also contains preservatives.

Test tubes 12 -16 mm in diameter or sample cups (Cat. No. AU1063).

Olympus HDL-Cholesterol Calibrator (Cat. No. ODC0023)

Precautions:

1. The Olympus HDL-Cholesterol Assay and Calibrator are for *in vitro* diagnostic use.
2. Do not ingest reagents or calibrators. Harmful if swallowed.
3. The HDL Calibrator contains sodium azide as a preservative, which may react with lead and copper plumbing to form highly explosive metal azides. If waste is discarded down the drain, flush it with a large volume of water to prevent azide buildup.
4. The HDL Calibrator is manufactured from human serum. WARNING: POTENTIAL BIOHAZARDOUS MATERIAL. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. All products derived from human blood should be treated as potentially infectious. Therefore adequate safety precautions are recommended.

Preparation:

The Olympus system HDL-Cholesterol reagent is ready to use. No preparation is needed.

For the HDL-C Calibrator, accurately add 1.0 mL of deionized water to the calibrator vial. Mix thoroughly. Avoid foaming.

Storage Requirements:

1. The unopened reagents and calibrators are stable until the expiration date printed on the label when stored at 2 - 8°C.
2. Opened reagents are stable for 30 days when stored in the refrigerated compartment of the analyzer.
3. The reconstituted calibrator is stable for 14 days when stored 2 - 8°C. It may be aliquoted and frozen once. The frozen reconstituted calibrator is stable for 30 days at ≤ -70°C.
4. Do not use reagents that have been frozen.
5. Protect the reagents from direct sunlight.

Indications of Deterioration

Unexpected discoloration of the reagent or calibrator, visible signs of microbial growth, turbidity or precipitation in reagent or calibrator may indicate degradation and warrant discontinuance of use.

Performance Parameters:
The following data was obtained using the Olympus system HDL-Cholesterol Reagent on Olympus AU400®/AU400e®, AU600®, AU640®/AU640e®, AU680®, AU2700®, and AU5400® analyzers according to established procedures.

Precision:

Estimates of precision, based on CLSI recommendations[10], are consistent with typical performance. The within run precision is less than 3% and total precision is less than 5%. Assays of control sera were carried out and data reduced following CLSI guidelines.

AU400®/AU400e® Results	**N=60**	**Within run**		**Total**	
	Mean, mg/dL	**SD**	**CV%**	**SD**	**CV%**
	38.16	0.56	1.47	1.34	3.51
	65.68	1.03	1.56	1.89	2.88
	82.08	1.03	1.25	2.90	3.53

Comparison:
Serum:

Patient samples were used to compare the Olympus System HDL-Cholesterol Reagent on the AU640®/AU640e® to another Olympus HDL-Cholesterol method (Method 2). Further studies were conducted as outlined below.

Y Method	**AU640®/ AU640e®**	**AU400®/ AU400e®**	**AU2700®/AU5400®/A U680e®**
X Method	**Method 2**	**AU640®/ AU640e®**	**AU640®/AU640e®**
Slope	1.07	1.01	1.01
Intercept	-1.2	-0.5	0.07
Correlation Coeff. (r)	0.991	0.998	0.998
No. of Samples (n)	115	115	115
Range (mg/dL)	24 - 89	23 - 91	25 - 89

Sensitivity:

Typical change in absorbance for 1 mg/dL of HDL-Cholesterol is 1.0 mAbsorbance.

Calibration:

Perform a one-point calibration (AB) using a water blank (blue rack) and the Olympus HDL Cholesterol Calibrator (Cat No. ODC0023). Calibration stability is 7 days. Validate the calibration by running

controls (see Quality Control section). Refer to analyzer User's Guide or Method Parameter Sheets for instrument settings.

Note: This calibrator has not been tested for use with any other Chemistry System other than those mentioned in this protocol.

Calibration Procedure:

Recalibration is required when any of the following conditions occur:

1. A reagent lot number has changed or there is an observed shift in control values.
2. Major preventative maintenance was performed on the analyzer.
3. A critical part was replaced.

Quality Control:

During operation of the Olympus analyzer at least two levels of an appropriate lipid control material should be tested a minimum of once a day. In addition, controls should be performed after calibration, blanking, with each new lot of reagents, and after specific maintenance or troubleshooting steps described in the appropriate Olympus User's Guide. Quality control

testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Analyzer Parameters:

A complete list of test parameters and operating procedures can be found in the appropriate User's Guide.

Calculations:

For SI Units (mmol/L), multiply the results by 0.0258.

Reporting Results:

Reference Ranges:

Olympus obtained Adult ranges[9]: 23 - 92 mg/dL

The NCEP guidelines classify HDL- C levels as follows:

1. < 40 mg/dL as indicative of a major risk factor for Coronary Heart Disease.
2. > 60 mg/dL as a negative risk factor for Coronary Heart Disease.

Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Procedures for Abnormal Results:

Abnormal results are flagged by the listed analyzers according to the normal values entered by the user into the instrument parameters.

Reporting Format:

Results are automatically printed out for each sample in mg/dL at 37^{O}C.

Limitations:

The Olympus HDL-Cholesterol procedure is linear from 2.5 mg/dL to 200 mg/dL. Samples exceeding the upper limit of linearity should be diluted with physiological saline and repeated and the result multiplied by the dilution factor. Sample may be diluted, repeated and multiplied by the dilution factor automatically utilizing the AUTO REPEAT RUN.

Endogenous triglyceride levels gave acceptable performance up to 2000 mg/dL. Samples with triglyceride levels > 2000 mg/dL should be diluted.

Carryover from this Olympus HDL Cholesterol reagent to Olympus Lipase reagent may results in elevated lipase values. Please refer to the Contamination Parameter Tab in the User Guide for proper programming on your AU system.

Interfering Substances:

Results of studies[7] conducted show that the following substances interfere with this HDL-Cholesterol procedure.

For AU400®/AU400e®

Ascorbate:	Interference less than 3% up to 20 mg/dL Ascorbate
Bilirubin:	Interference less than 3% up to 40 mg/dL conjugated Bilirubin
	Interference less than 10% up to 40 mg/dL unconjugated Bilirubin
Globulin:	Interference less than 5% up to 5 g/dL added Gamma-Globulin
Hemolysis:	Interference less than 10% up to 500 mg/dL Hemolysate
Lipemia:	Interference less than 5% up to 1500 mg/dL Intralipid*
Triglyceride:	Interference less than 10% up to 900 mg/dL Triglyceride**

* Intralipid®, manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

** Triglyceride concentrate, manufactured by Miles Pentex, Cat No. 96-051-6, was used to measure triglyceride interference. No significant interference was observed from samples containing native triglycerides up to 1000 mg/dL.

Note: There is poor correlation between lipemia and triglyceride concentration.

The information presented is based on results from Olympus studies and is current at the date of publication. Olympus America Inc. makes no representation about the completeness or accuracy of results generated by future studies. For further information on interfering substances, refer to Young[8] for a compilation of reported interferences with this test.

In very rare cases gammopathy, especially monoclonal IgM (Waldestrom's macroglobulinemia), may cause unreliable results.

References:

1. NIH Consensus Conference: Triglyceride, High-density Lipoprotein and Coronary Heart Disease. JAMA 1993; 269: 505-10.
2. Wiebe, D.A, Warnick, G.R., Measurement of High-density Lipoprotein Cholesterol Concentration. In Rifai N., Warnick G.R., eds. *Laboratory Measurement of Lipids, Lipoproteins and Apolipoproteins*. Washington, DC: AACC Press, 1994: 91-105.
3. Badimon, U.U., *et al.* Regression of atherosclerotic lesions by high- density lipoprotein plasma fraction in the cholesterol-fed rabbit. J.Clin. Invest. 1990; 85:1234-1241.
4. Warnick, G.R., *et al*, National Cholesterol Education Program recommendations for Measurement of High Density Lipoprotein Cholesterol: Executive Summary Clin. Chem. 1995; 41:1427-1433.
5. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). The Expert Panel, JAMA, 2001; 285:2486-97.
6. Tietz, *Textbook of Clinical Chemistry, Third Edition*, W.B. Saunders, 1999, 849.

2.2.5. Glucose

Principle:

Serum glucose levels may be abnormally high (hyperglycemia) or abnormally low (hypoglycemia)[1]. Glucose measurements are used in the diagnosis and treatment of carbohydrate metabolism disorders including diabetes mellitus, neonatal hypoglycemia, and idiopathic hypoglycemia, and of pancreatic islet cell carcinoma.

Glucosuria (the presence of urinary glucose) is common in healthy, pregnant women. The cardinal feature of the glucosuria of pregnancy is a conspicuous variability both from day to day and during the course of the day.[2] Glucose is not present in normal patient urine.

Determinations of cerebrospinal fluid (CSF) glucose help distinguish bacterial from viral meningitis; the glucose value is often low (less than 40% to 45% of simultaneously analyzed, equilibrated serum glucose) in bacterial meningitis and tuberculous meningitis and is generally normal in viral disease. Carcinomatous meningitis (widespread infiltration of the meninges by tumor cells) also drives CSF glucose values below the normal range.[2]

Intended Use:

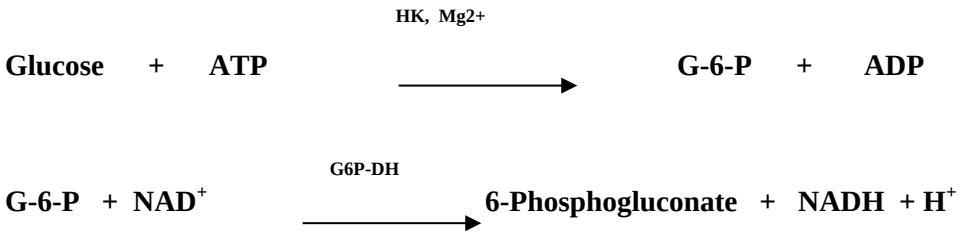
System reagent for the quantitative determination of Glucose in human serum, plasma, urine or cerebrospinal on OLYMPUS analyzers.

Glucose reagent OSR6621 is for use on the AU2700® and AU5400® analyzers only.

Methodology:

Stein[1] first introduced the hexokinase G-6-PDH method for assay of glucose in serum or plasma. Several investigators[3,4,5,6] have demonstrated the accuracy and usefulness of the method.

In this Olympus procedure, glucose is phosphorylated by hexokinase (HK) in the presence of adenosine triphosphate (ATP) and magnesium ions to produce glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). Glucose-6-phosphate dehydrogenase (G6P-DH) specifically oxidizes G-6-P to 6-phosphogluconate with the concurrent reduction of nicotinamide adenine dinucleotide (NAD^+) to nicotinamide adenine dinucleotide, reduced (NADH). The change in absorbance at 340/380 nm is proportional to the amount of glucose present in the sample.



Specimen:

Patient Preparation:

Prior to sample collection, an 8-12 hour fast is recommended but not required for the patient.

Additional instructions for patient preparation as designated by this laboratory:
ACRL require 12 hour fast.

Type:

Fasting serum or plasma (EDTA, heparin, or sodium fluoride) samples, free from hemolysis, are the recommended specimens. Separate from red cells rapidly to minimize loss of glucose through glycolysis.

Fresh, random collections are recommended for urine specimens.

Additional type conditions as designated by this laboratory:
EDTA plasma is preferred

Handling Conditions:

Glucose in serum, free from hemolysis and bacterial contamination, and without added preservatives, is stable for 8 hours when stored at room temperature (15-25°C), or 72 hours when stored refrigerated at 2-8°C.[7] Fluoride preserved plasma samples are stable for 24 hours at 25°C.

Urine specimens should be maintained at 2 - 8°C and analyzed as soon as possible.[7]

Cerebrospinal fluid can be stored between 2 - 8°C for at least 5 days if protected from evaporation. Specimens that will not be tested within 5 days should be stored frozen at ≤-20°C immediately after collection.[2]

Additional handling conditions as designated by this laboratory:
Real time storage = refrigerated 2-8°C up to 19 days
Short term freeze = ≤ - 20°C up to 1 month
Long term freeze = ≤ -80°C (length not determined)

Equipment And Materials:
Equipment:

Beckman Coulter Olympus AU400e analyzer.

Materials:

Olympus System Glucose Reagent

Final concentration of reactive ingredients:

PIPES- buffer (pH 7.6)	24.0	mmol/L
NAD+	≥ 1.32	mmol/L
Hexokinase	≥ 0.59	KU/L
ATP	≥ 2.0	mmol/L
Mg^{2+}	2.37	mmol/L
G6P- DH	≥ 1.58	KU/L

Also contains preservatives.

Reagent storage location in this laboratory:

Walk-in cold room in F-740

Test tubes 12 -16 mm in diameter or sample cups (Cat No. AU1063).

Storage location of test tubes or sample cups in this laboratory:

Rm. F-740 closet

Preparation:

The Olympus System Glucose reagents are liquid, ready for use. No preparation is needed.

Storage Requirements:

1. The unopened reagents are stable until the expiration date printed on the label when stored at 2 - 8°C.
2. Opened reagents are stable for 30 days when stored in the refrigerated compartment of the analyzer.
3. Unreconstituted calibrator and diluent are stable until the expiration date stated on the label when stored at 2 - 8°C.
4. For Glucose Unreconstituted calibrator and diluent are stable until the expiration date stated on the label when stored at 2 - 8°C e, reconstituted calibrator materials are stable for 7 days from the date of reconstitution when stored at 2 - 8°C. The materials should be capped and stored upright 2 - 8°C when not in use.

Performance Parameters:

The following data was obtained using the Olympus System Glucose Reagent on an Olympus AU400®/AU400e®, AU600®, AU640®/AU640e®, AU680®, AU2700®, and AU5400® analyzers according to established procedures.

Precision:

Estimates of precision, based on CLSI recommendations[10], are consistent with typical performance. The within run precision for serum samples is less than 3% and total precision is less than 3%. Assays of control sera were performed and this data reduced following CLSI guidelines.

Serum:					
AU400®/AU400e® Results	**N=60**	**Within run**		**Total**	
	Mean, mg/dL	**SD**	**CV%**	**SD**	**CV%**
	55.87	0.536	0.96	0.971	1.74
	114.14	0.982	0.86	1.512	1.27
	310.93	2.401	0.77	3.779	1.22

Urine:					
AU400®/AU400e® Results	**N=60**	**Within run**		**Total**	
	Mean, mg/dL	**SD**	**CV%**	**SD**	**CV%**
	18.07	0.279	1.55	0.289	1.60

Comparison:
Serum:

Patient samples were used to compare the Olympus System Glucose Reagent on the AU600® to another Olympus analyzer Glucose method (Method 2). Further studies were conducted as outlined below.

Y Method	**AU600®**	**AU400®/ AU400e®**	**AU640®/ AU640e®**	**AU2700®/AU5400®/ AU680®**
X Method	**Method 2**	**AU600®**	**AU600®**	**AU640®/AU640e®**
Slope	0.99	1.02	0.986	0.9873
Intercept	-5.6	0.10	0.4	-0.3
Correlation Coeff. (r)	0.998	0.99	1.000	0.9997
No. of Samples (n)	120	120	180	283
Range (mg/dL)	3.5 – 654.0	43 - 620	10 - 644	12 – 547

Urine:

Urine samples were used to compare the Olympus System Glucose Reagent on the AU600® to another Olympus analyzer Glucose (Method 2). Further studies were conducted as outlined below.

Y Method	**AU600®**	**AU400®/ AU400e®**	**AU640®/ AU640e®**	**AU2700®/AU5400®/ AU680®**
X Method	**Method 2**	**AU600®**	**AU600®**	**AU640®/AU640e®**
Slope	1.04	1.01	0.996	1.007
Intercept	-2.4	-0.7	0.8	0.9
Correlation Coeff. (r)	0.9978	0.9998	0.9997	0.9999
No. of Samples (n)	120	86	96	90
Range (mg/dL)	0 - 308	10 - 613	2 - 1099	11 – 708

Sensitivity:

Typical change in absorbance per minute for 1 mg/dL of Glucose is 2.0 mAbsorbance in the Olympus AU400®/AU400e®, AU600®, AU640®/AU640e®, and AU680® analyzers and 2.5 mAbsorbance in the AU2700®, and AU5400® analyzers.

Calibration:
Standard Preparation:

Perform a one-point calibration (AB) using a water blank (blue rack) and the appropriate calibrator in a yellow calibration rack. The frequency of calibration is every 30 days. Calibration of this glucose procedure is accomplished by use of the Olympus Chemistry Calibrator (Cat No. DR0070), which is traceable to the National Institutes of Standards and Technology (NIST) Standard Reference Material (SRM) 909b.

Calibration Procedure:

Recalibration is required when any of the following conditions occur:

1. A reagent lot number has changed or there is an observed shift in control values.
2. Major preventative maintenance was performed on the analyzer.
3. A critical part was replaced.

Quality Control:

During operation of the Olympus analyzer at least two levels of appropriate control material should be tested a minimum of once a day. In addition, controls should be performed after calibration, with each new lot of reagents, and after specific maintenance or troubleshooting steps described in the appropriate Olympus User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Appropriate qualified urine controls such as Bio-Rad urine controls, should be established and utilized during urine analysis.

Reporting Results:

Reference Ranges:
Serum[6]:

Adult	70 - 105	mg/dL
Newborn	21 - 58	mg/dL

Urine:

There should be no detectable glucose in urine.

Cerebrospinal fluid:

Child	60 - 80 mg/dL
Adult	40 - 70 mg/dL

Limitations:

The Olympus Glucose procedure is linear from 10 - 800 mg/dL for serum determinations; 10 - 700 mg/dL for urine determinations; and 0 – 360 mg/dL for cerebrospinal fluid determinations. Samples exceeding the upper limit of linearity should be diluted and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically by utilizing the AUTO REPEAT RUN.

Interfering Substances:

Results of studies[8] conducted on the AU400®/AU400e®, AU600®, AU640®/AU640e®, AU680®, AU2700®, and AU5400® show that the following substances interfere with this glucose procedure.

For AU400®/AU400e®

Bilirubin:	Interference less than 2% up to 40 mg/dL Bilirubin
Hemolysis:	Interference less than 2% up to 500 mg/dL Hemolysate
Lipemia:	Interference less than 2% up to 1000 mg/dL Intralipid*

* Intralipid, manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.

References:

1. Stein, M.W., Clinical Methods of Enzymatic Analysis, Academic Press, 117, 1965.
2. Kaplan, L.A. and Pesce, A.J., Clinical Chemistry Theory, Analysis and Correlation, C.V. Mosby., St. Louis, 1989.
3. Neely, W.E., Clin Chem 18: 509, 1972.
4. Keller, D.M., Clin Chem 11: 471, 1965.
5. Yee, H.Y., Clin Chem 17: 648, 1971.
6. Bondar, R.J.L. and Mead, D.C., Clin Chem, 20: 586, 1974.
7 Tietz, N.W.(ed), Clincial Guide to Laboratory Tests, Second Edition, W.B. Saunders, 1990.
8. CLSI/NCCLS, Interference Testing in Clinical Chemistry, EP7-A, 2002.

Young, D.S., Effects of Drugs on Clinical Laboratory Tests, AACC Press, Fifth Edition, 2000.

10. CLSI/NCCLS Evaluation Protocol EP5-A, 1999.

2.2.6 Ultra C-Reactive Protein

Intended Use

For the quantitative determination of C-reactive protein in serum by latex particle enhanced immunoturbidimetric assay. Measurement of C-reactive protein aids in evaluation of the amount of injury to body tissues.

For *In Vitro* Diagnostic Use.

Introduction

C-reactive protein (CRP) is described in the literature as an acute phase protein that is involved in the activation of complement, acceleration of phagocytosis, and detoxification of substances released from damaged tissue. CRP is one of the most sensitive indicators of inflammation.

In response to an inflammatory stimulus, a rise in CRP may be detected within 6 hours. CRP is a sensitive, non-specific indicator of acute phase reactants[1,2,3]. The level of CRP in serum is elevated in patients with arthritis or liver disease such as hepatitis A, hepatitis B, or biliary cirrhosis, and after severe infections such as septic shock.

The CRPUltra is intended for the quantitative determination of human CRP by latex particle enhanced immunoturbidimetric assay (ITA). ITA methods for quantitative determination of antibody and antigen immunoprecipitation complexes have been described[4,5,6,7].

Principle Of Test
Latex particles coated with antibody specific to human CRP aggregate in the presence of CRP from the sample forming immune complexes. The immune complexes cause an increase in light scattering which is proportional to the concentration of CRP in the serum. The light scattering is measured by reading turbidity at 572 nm. The sample CRP concentration is determined versus dilutions of a CRP standard of known concentration.

Equipment

Beckman Coulter Olympus AU400e analyzer.

Reagents (Liquid stable)

R-1: Buffer Reagent 1 x 30 ml

170 mM Glycine buffer solution

R-2: Latex Suspension 1 x 20 ml

0.17% (w/v) solution of latex particles

coated with rabbit anti-human CRP antibodies

Warnings And Precautions
FOR *IN VITRO* DIAGNOSTIC USE.

Not to be used internally in humans or animals. Normal precautions for handling laboratory reagents should be followed.

Do not mix or use reagents from one test kit with those from a different lot number. Do not use reagents past their expiration date stated on each reagent container label.

Do not pipette by mouth. Avoid ingestion and contact with skin.

Reagents in this kit contain < 0.1 w/v% sodium azide as a preservative. Sodium azide may form explosive compounds in metal drain lines. When disposing of reagents through plumbing fixtures, flush with copious amounts of water.

Reagent Preparation
Reagents are ready to use and do not require reconstitution. Mix gently before using.

Storage and Stability
All reagents should be stored at 2-10°C and protected from light. Unopened reagents can be used for one year from the date of manufacture as indicated on the expiration date on the package and bottle labels. Once the reagent vial has been opened, store tightly capped at 2-10°C and use within 1 month.

Interference
Hemoglobin, Lipid (Triglycerides), and Bilirubin do not interfere with the latex particle enhanced immunoturbidimetric determination of C-reactive protein.

Dust particles or other particulate matter in the reaction solution may result in extraneous light-scattering, which may affect the accuracy of this test.

Specimen Collection And Preparation
Serum test samples must be collected in the manner routinely used for clinical laboratory tests. Freshly drawn serum is preferred and should be used within the day of collection. Samples may also be stored refrigerated (2-10°C) for one week or at -30°C for up to 1 year. Use undiluted samples for this assay.

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Use plastic tubes for storing the sample, do not use glass.

Calibration Curve
It is recommended that a multi-point calibration curve be made using either the CRPUltra Multi-Calibrator Set A (standard protocol) or CRPUltra Multi-Calibrator Set B (high sensitivity protocol). Please be sure to use the proper instrument application for the calibrator you select. It is recommended that the user determine calibration frequency as this will depend on the instrument and type/number of other assays being run. Initially, calibration should be performed each day.

Quality Control
It is recommended that commercially available control serum with a known concentration of CRP be included in all assay runs. The Lipid Laboratory uses the hs-CRP 3 x 2 mL control set; Catalog numbers are: Level 1 (18402), Level 2 (18404), Level 3 (18406). These controls represent low, normal and high concentrations of CRP.

Results
Calculations
CRP levels are determined by the autoanalyzer using the prepared calibration curve. CRPUltra Rev. 9/2001 2

Limitation of Procedure
Standard Protocol: The CRPUltra has a measurable range from 10.0 to 2000 µg/dl (0.01 to 2.00 mg/dl) using the CRP Multi-Calibrator Set A and standard parameters.

High Sensitivity Protocol: The CRPUltra has a measurable range from 5.0 to 1000 µg/dl (0.005 to 1.000 mg/dl) using the CRP Multi-Calibrator Set B and high sensitivity parameters.

Reagents should not be used after the expiration date indicated on the kit label. Do not mix reagents with different lot numbers.

If the CRP concentration is greater than highest calibrator value, dilute one part sample with four parts isotonic saline and re-assay. Multiply results by 5 to compensate for the dilution.

Performance
The following performance data was obtained using a Hitachi 717 analyzer and standard protocol.

Sensitivity:

When saline is used as a sample, the range of absorbance change per minute is -0.0050 to 0.0050, while a standard CRP solution containing 1000 µg/dl is 0.0650 to 0.1000 after subtracting the saline blank.

Specificity:

When serum containing a known level of CRP (250 µg/dl) is measured, the assay value obtained is within ± 10%.

Precision

Samples tested were commercial human CRP control serum.

Precision Assay:

(Within-run)

Sample I Sample II Sample III

N=20 N=20 N=20

Mean=2.74 mg/L Mean=8.64 mg/L Mean=17.43 mg/L

SD=0.034 SD=0.068 SD=0.135

CV=1.24% CV=0.78% CV=0.77%

Precision Assay:

(Between-run)

CRP values were tested on 3 days.

Sample I Sample II Sample III

N=10 N=10 N=10

Mean=2.65 mg/L Mean=8.55 mg/L Mean=17.13 mg/L

SD=0.111 SD=0.092 SD=0.204

CV=4.2% CV=1.08% CV=1.19%

Assay Range

Standard Protocol: 10.0 - 2000 µg/dl or

(0.1 - 20 mg/L)

High Sensitivity Protocol: 5.0 - 1000 µg/dl or

(0.05 - 10 mg/L)

Lower Limit of Detection

Standard Protocol: 10 µg/dl (0.10 mg/L)

High Sensitivity Protocol: 5 µg/dl (0.05 mg/L)

Correlation

y= 1.034x - 0.173

r= 0.999

x= Company A's latex CRP nephelometric assay

y= CRPUltra

Interference

Bilirubin C: No interference up to 60 mg/dl.

Bilirubin F: No interference up to 60 mg/dl.

Hemoglobin: No interference up to 500 mg/dl.

Lipid: No interference up to 1500 mg/dl triglycerides.

Expected Values

Expected value for CRP in healthy individuals is from 0.007 to 0.494 mg/dl. This value was calculated using 496 healthy adults. It is recommended that each laboratory establish its own expected range.

References

1. Osmond, A.P., et al. Proc. Natl. Acad. Sci. 74:739-743, 1977.

2. Pepys, M.B. Lancet. 1:653-657, 1981.

3. Schultz, D.R. and P.I. Arnold. Semin. Arthritis Rheum. 20(3): 129-147, 1990.

4. Killingsworth, L.M. and J. Savory. J. Clin. Chem. 19:403-407, 1973.

5. Lizana, J. and K. Helling. Clin. Chem. 20:1181, 1974.

6. Otsuji, S., et al. Clin Chem. 28:2121-2124, 1982.

7. Malkus, H., et al. Clinica Chimica Acta, 88:523-530, 1978.

2.2.7 Cystatin C

Summer 2012: assay for Cystatin C changed to Gentian, and analysis moved to Chemistry Lab. All Visit 5 samples to receive Cystatin C analysis at MN.

Intended Use

For the quantitative measurement of cystatin C concentration in human serum, heparinized plasma, or EDTA plasma. Cystatin C measurements are used as an aid in the diagnosis and teatment of renal disease.

Summary

Reagents must be used with the Genzyme Cystatin C Calibrator. Caution: Avoid freezing reagents
Caution: Reagent 1 and 2 contain <0.1% sodium azide as an anti microbial agent. Sodium azide may react with lead and copper plumbing to form a potentially explosive metal azide build-up. Flush with copious amounts of water when discarding material.

Equipment

Beckman Coulter Olympus AU400e analyzer.

Reagent Preparation

Reagent 1: Liquid. Mix contents gently, prior- to use.
Reagent2: Liquid. Mix thoroghly by inverting the bottle a minimum of 20 times until the colloidal qold particles become a homogeneous suspension lnvert daily.

Storage And Stability

Un-opened reagent is stable until the expiration date shown on the label when stored at 2 - 8°C.
Once opened, the reagent is stable for up to one month at 2 - 8°C.
DO NOT FREZE,

lndications of Deterioration
Presence of turbidity or microbial growth may indicate deterioration causing an inability to recover contol values.

Specimen Collection And Preparation

Serum, EDTA plasma, or lithium heparinized plasma. Are the recommended sample types. Use standard sample collection and preparation methods.[12]
lf not analyzed promptly, serum or plasma specimens may be stored at 2-8°C for 19 days, or at 20 - 25°C for 4 days. If storage periods will be longer, the specimens
should be frozen at -20'C. Samples may be frozen and thawed twice.

Procedure

Materials Provlded
Gen4mes Cystatjn C.reagents 1 and 2 are required for the measurement of cystatin C,

Limitations / Lntetrering Substances

All lnteference studies were conducted according to CLSI EP7 and WHO standards.

Expected Values

Samples from 196 apparently healthy individuals (101 males and 95 females) ranqing in age from 18-79 were tested with the Genzyme Cystatin C assay using CLSI C2816. The results, calculated non-parametically, were in the range 0.61-1.17 mg/L.

References

1.0 Package insert**,** Genzyme Corporation Framingham, MA 01701-9322 USA Phon6i 800,3321042.

2.2.8 INSULIN

Intended Use

Immunoassay for the in vitro quantitative determination of human insulin in human serum and plasma. The determination of insulin is utilized in the diagnosis and therapy of various disorders of carbohydrate metabolism, including diabetes mellitus and hypoglycemia.
The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Insulin is a peptide hormone with a molecular weight of approximately 6000 daltons. It is secreted by the B-cells of the pancreas and passes into circulation via the portal vein and the liver. Insulin is generally released in pulses, with the parallel glucose cycle normally about 2 minutes ahead of the insulin cycle.

The insulin molecule consists of two polypeptide chains, the α-chain with 21 and the β-chain with 30 amino acids. Biosynthesis of the hormone takes place in the β-cells of the islets of Langerhans in the form of single-chain preproinsulin, which is immediately cleaved to give proinsulin. Specific proteases cleave proinsulin to insulin and C-peptide which pass into the bloodstream simultaneously. About half of the insulin, but virtually none of the C-peptide, is retained in the liver. Circulating insulin has a half-life of 3–5 minutes and is preferentially degraded in the liver, whereas inactivation or excretion of proinsulin and C-peptide mainly takes place in the kidneys.

The amino acid sequence of insulin has remained surprisingly constant during evolution, with the result that prior to the development of genetically engineered human insulin it was possible to successfully use porcine or bovine insulin in the therapy of diabetes mellitus.

The action of insulin is mediated by specific receptors and primarily consists of facilitation of the uptake of sugar by the cells of the liver, fatty tissue and musculature; this is the basis of its hypoglycemic action.

Serum insulin determinations are mainly performed on patients with symptoms of hypoglycemia. They are used to ascertain the glucose/insulin quotients and for clarification of questions concerning insulin secretion, e.g. in the tolbutamide test and glucagon test or in the evaluation of oral glucose tolerance tests or hunger provocation tests.

Although the adequacy of pancreatic insulin synthesis is frequently assessed via the determination of C-peptide, it is still generally necessary to determine insulin. For example, therapeutic administration of

insulins of non-human origin can lead to the formation of anti-insulin antibodies. In this case, measurement of the concentration of serum insulin shows the quantity of free - and hence biologically active - hormone, whereas the determination of C-peptide provides a measure of the patient's total endogenous insulin secretion.

A disorder in insulin metabolism leads to massive influencing of a number of metabolic processes. A too low concentration of free, biologically active insulin can lead to the development of diabetes mellitus. Possible causes of this include destruction of the β-cells (type I diabetes), reduced activity of the insulin or reduced pancreatic synthesis (type II), circulating antibodies to insulin, delayed release of insulin or the absence (or inadequacy) of insulin receptors.

On the other hand, autonomous, non-regulated insulin secretion is generally the cause of hypoglycemia. This condition is brought about by inhibition of gluconeogenesis, e.g. as a result of severe hepatic or renal failure, islet cell adenoma, or carcinoma. Hypoglycemia can, however, also be facilitated intentionally or unintentionally (factitious hypoglycemia).

In 3% of persons with reduced glucose tolerance, the metabolic state deteriorates towards diabetes mellitus over a period of time. Reduced glucose tolerance during pregnancy always requires treatment. The clearly elevated risk of mortality for the fetus necessitates intensive monitoring.
The Elecsys Insulin assay employs two monoclonal antibodies which together are specific for human insulin.

Test Principle

Sandwich principle. Total duration of assay: 18 minutes.
1st incubation: Insulin from 20 µL sample, a biotinylated monoclonal insulin-specific antibody, and a monoclonal insulin-specific antibody labeled with a ruthenium complex[a] form a sandwich complex. 2nd incubation: After addition of streptavidin-coded microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Specimen Collection And Handling

Only the specimens listed below were tested and found acceptable. Serum collected using standard sampling tubes or tubes containing separating gel. Li-heparin, K_3-EDTA, and sodium citrate plasma. Hemolysis interferes, as insulin-degrading peptidases are released from erythrocytes. Criterion: Recovery within 90-110% of serum value or slope 0.9-1.1 + intercept within < ± 2 x analytical sensitivity (LDL) + coefficient of correlation > 0.95.
Stable for 24 hours at 2-8°C, 6 months at -20°C. Freeze only once.
The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide.
Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25°C) before measurement.
Because of possible evaporation effects, samples, calibrators, and controls on the analyzers should be measured within 2 hours.

Materials And Equipment Required
Test Instrument:
Elecsys 1010 / 2010 / **MODULAR** *ANALYTICS* E170 systems / **cobas e** 411 **/ cobas e** 601 analyzers

Materials Provided

Elecsys Insulin kit Cat. No. 12017547122 100 tests

Materials Required (But Not Provided)

- Cat. No. 12017504122, Insulin CalSet, for 4 x 1 mL
- Cat. No. 03609979190, PreciControl MultiAnalyte, for 2 x 2 mL each of PreciControl MultiAnalyte 1 and 2 or Cat. No. 11731416160, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal
1 and 2
- General laboratory equipment

Reagents – Working Solutions

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles, 0.72 mg/mL; preservative.

R1 Anti-insulin-Ab~biotin (gray cap), 1 bottle, 10 mL: Biotinylated monoclonal anti-insulin antibody (mouse) 1 mg/L; MES buffer 50 mmol/L, pH 6.0; preservative.

R2 Anti-insulin-Ab~Ru(bpy)$_3^{2+}$ (black cap), 1 bottle, 10 mL: Monoclonal anti-insulin antibody (mouse) labeled with ruthenium complex 1.75 mg/L; MES buffer 50 mmol/L, pH 6.0; preservative.

Storage And Stability

Store at 2-8°C.
Store the Elecsys Insulin reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.
Stability:

unopened at 2-8°C: up to the stated expiration date after opening at 2-8°C: 12 weeks
on **MODULAR** *ANALYTICS* E 170 system and **cobas e** 601 analyzer: 4 weeks
on Elecsys 2010 system and **cobas e** 411 analyzer: 4 weeks

Calibration

Traceability: This method has been standardized using the 1st IRP WHO Reference Standard 66/304 (NIBSC). Every Elecsys Insulin reagent set has a barcoded label containing the specific information for

calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys Insulin CalSet system.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:
MODULAR *ANALYTICS* E 170, Elecsys 2010 systems and **cobas e** analyzers:
- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)

For all analyzers:
- as required: e.g. quality control findings outside the specified limits

Refer to the appropriate calibration section of your Operator's Manual for specific calibration instructions.

Quality Control

For quality control, use Elecsys PreciControl MultiAnalyte 1 and 2 or Elecsys PreciControl Universal 1 and 2 systems.Other suitable control material can be used in addition.
Controls for the various concentration ranges should be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration. The control intervals and limits should be adapted to each laboratory's individual requirements.
Values obtained should fall within the defined limits.
Each laboratory should establish corrective measures to be taken if values fall outside the limits.
Please note: Commercial controls may contain insulin of animal origin.
When assessing results, the corresponding cross-reactivity of this test must be taken into account; see under "Analytical specificity".

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in µU/mL or pmol/L).

Conversion factors: µU/mL x 6.945 = pmol/L
pmol/L x 0.144 = µU/mL

Procedure

Refer to the appropriate Operator's Manual for Procedure Step-by-Step Instructions.

Reagent Handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.
All information required for correct operation is read in via the respective reagent barcodes.
Assay For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.
Resuspension of the microparticles takes place automatically before use.

Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

MODULAR *ANALYTICS* E 170, Elecsys 2010 systems and **cobas e** analyzers: Bring the cooled reagents to approx. 20°C and place on the reagent disk (20°C) of the analyzer. Avoid the formation of foam. The system **automatically** regulates the temperature of the reagents and the opening/closing of the bottles.
Elecsys 1010 analyzer: Bring the cooled reagents to approx. 20-25°C and place on the sample/reagent disk of the analyzer (ambient temperature 20-25°C). Avoid the formation of foam. **Open** bottle caps **manually** before use and **close manually** after use. Store at 2-8°C after use.

2.2.9 TROPONIN T HS

(Troponin T hs (high sensitive) Under Development and FDA Clearance)

2.2.10 NT-PROBNP

Instruments

Cobas e411

Intended Use

lmmunoassay for the in vitro quantitative determination ol N-terminal pro-Brain natriuretic peptide in human serum and plasma. The Elecsys proBNP ll assay is used as an aid in the diagnosis of individuals suspected of having congestive heart failure. The test is further indicated for the risk stratification of patients with acute coronary syndrome and congestive
heart failure. The test may also serve as an aid in the assessment of increased risk of cardiovascular events and mortality in patients at risk for heart failure who have stable coronary artery disease, the electrochemiluminescence immunoassay "ECLIA' is intended for
use on Elecsys and cobas e immunoassay analyzers.

Summary

Left ventricular dysfunction can occur as a part of coronary heart disease, arterial hypertension, valvular disease, and primary myocardial disease. lf the left ventricular dysfunction remains untreated and is progressive, the potential for mortality is high, e.g. due to sudden cardiac death.
Chronic cardiac insufficiency is a clinical syndrome caused by impairment of the cardiac pumping function. Based on the symptoms, the severity of cardiac insufficiency is classified in stages (New York Heart Association classification INYHA] l-lv).1 2 Clinical information and imaging procedures are used to diagnose lelt ventricular dysfunction.3 The significance of natriuretic peptides in the control of cardiovascular system function has been demonstrated. Studies reveal that natriuretic peptides can be used for diagnostic clinical problems associated with left ventricular dysfunction.

Test Principle

Sandwich principle. Total duration of assay: 18 minutes. . 1st incubation: Antigen in the sample (15;), a biotinylated monoclonal NT-proBNP-specific antibody, and a monoclonal NT-proBNP-specific antibody labeled with a ruthenium complexa form a sandwich complex.
2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin. . The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell, Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. . Results are determined via

a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.
a) Tris(2,2'-bipyridyl-ruthenium(ll)-complex (Ru(bpy)3*)

Reagents - Working Solutions

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.
R1 Anti-NT-proBNP-Ab-biotin (gray cap), 1 bottle, 9 mL:
Biotinylated monoclonal anti-NT-proBNP antibody (mouse) 1.1 pg/mL;
phosphate buffer 40 mmol/1, pH 5.8; preservative.
B2 Anti-NT-proBNP-Ab-Ru(bpy)3. (black cap), 1 bottle, 9 mL:
-...* ""--"Monoclonal anli-NT-proBNP antibody (sheep) labeled with ruthenium
complex 1.1 pg/ml; phosphate bulfer 40 mmol/l, pH 5.8; preservative.

Precautions And Warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents. Disposal of all waste material should be in accordance with local guidelines. Safety data sheet available for professional user on request. Serum concentrations ol natriuretic peptides may be elevated in patients with acute myocardial infarction, patients that are candidates for renal dialysis, and patients that have undergone renal dialysis. The Elecsys proBNP ll test, like all laboratory tests, does not provide a delinitive diagnosis. As with all in vitro diagnostic tests, the test results should be interpreted by the physician in conjunction with other laboratory test results and patient clinical findings. Avoid the formation of foam with all reagents and sample types (specimens, calibrators, and controls).

Reagent Handling

The reagents in the kit have been assembled into a ready'for-use unit that cannot be separated.
All information required for correct operation is read in via the respective reagent barcodes.

Storage And Stability

Store at 2-8 "C.
Store the Elecsys proBNP ll reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use. Stability: proBNP Il Nterminal pro B-type natriuretic peptide

Specimen Collection And Preparation

Only the specimens listed below were tested and found acceptable. Serum collected using standard sampling tubes or tubes containing separating gel. Li-, NHa-heparin and K2-, K3-EDTA plasma.

Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within < t 2 x analytical sensitivity (LDL) + coelficient ol conelation > 0.95. Stable for 3 days at 20-25 "C,6 days18 at 2-8 "C, 24 months at -20 "C. The sample types listed were tested with a selection ol sample collection tubes that were commercially available at the time ol testing, i.e. not all available tubes oi all manufacturers were tested. Sample collection systems from various manufacturers may conlain diflering materials which could aflect the test results in some cases. When processing samples in primary tubes (sample collection

systems), follow the instructions of the tube manufacturer. Centrifuge samples containing precipitates before per(prming the assay.
Do not use samples and controls stabilized with azide. Ensure the patients' samples, calibrators, and controls are at ambient temperature (20-25 "C) be{ore measurement. Because of possible evaporation effects, samples, calibrators, and controls on lhe analyzers should be measured within 2 hours,

Materials Provided

See "Reagents - working solutions" section lor reagents.

Materials Required (But Not Provided)

. IREFI 04842472190, proBNP ll CalSet, for 4 x 1 mL
. IREFI 04917049160, PreciControl Cardiac ll, lor 2x2 mL each
of PreciControl Cardiac ll 1 and 2
. FEFI 03183971122, Diluent Universal, 2 x 36 mL sample diluent
.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.
Resuspension 0f the microparticles takes place automatically before use.
Read in the test-specific parameters via the reagent barcode. lf in exceptional cases the barcode cannot be read, enter the 1S-digit sequence of numbers.

Calibration

Traceability: This method has been standardized against the Elecsys proBNP assay. . This in turn was standardized against reference standards by weighing pure synthetic NT-proBNP
(1-76) into an equine serum matrix.
Every Elecsys proBNP ll reagent set has a barcoded label containing the specific information for calibration ol the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys proBNP ll CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows: . after 1 month (28 days) when using lhe same reagent lot
.after 7 days (when using the same reagent kit on the analyzer) .as required: e.g. quality control findings outside the specified limits

Quality Control

For qualily control, use Elecsys PreciControl Cardiac ll 1 and 2. Other suitable control material can be used in addition. Controls for the various concentration ranges should be run as single determinations at leasl once every 24 hours when the tesl is in use, once per reagent kit, and after every calibration, The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits. Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration oi each sample (either in pmol/L or pg/ml).

Conversion factors:

pmol/lx 9.457 = pg/ml
pg/m1x 0.118 = pmol/L

Limitations - Interference

The assay is unaffected by icterus (bilirubin < 428 pmol/L or < 25 mgidL), hemolysis (Hb < 0.621 mmol/L or < 1.0 g/dl), lipemia (intralipids < 171 mmol/L or < 1500 mg/dl), and biotin < 82 nmol/L or < 30 ng/ml.
ln patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours alter the last biotin administration.
No interference was observed from rheumatoid factors up to a concentration of 1500 lU/mL.
There is no high-dose hook effect at NT-proBNP concentrations up to 33400 pmol/L (300000 pg/ml).
ln vitto tests were performed on 51 commonly used pharmaceuticals. No interlerence with the assay was found. ln rare cases, interference due to extremely high titers of antibodies to analyte-specilic antibodies, streptavidin or ruthenium can occur. These ellects are minimized by suitable test design. For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits And Ranges

Measuring range

5.00-35000 pg/mL or 0.600-4130 pmol/L (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported as < 5.00 pg/ml (< 0.600 pmol/L). Values above the measuring range are reported as > 35000 pg/mL (> 4130 pmol/L) or up to 70000 pglnl (8277 pmol/L) for 2Jold diluted samples. Lower limits of measurement Limit of Detection (LoD), Limit of Quantitation (LoQ)

Limit of Detection = 5.00 pg/ml (0.600 pmolil)
Limit of Quantitation = 50.0 pg/ml (5.90 pmol/L)
The limit of detection was determined in accordance with the CLSI (Clinical and Laboratory Standards lnstitute) EP17-A requirements.

Dilution
Samples with NT-proBNP concentrations above the measuring range can be diluted with Elecsys Diluent Universal. The recommended dilution is 1:2 (either automatically by the MODULAR ANALYTTCS E170, Elecsys 2010 and cobas e analyzers or manually). The concentration of the diluted sample must be > 1770 pmol/L or > 15000 pg/ml. After manual dilution, multiply the result by the dilution factor. After dilution by the analyzers, the MODULAR ANALYTICS E170. Elecsys 2010 and cobas e software automatically takes the dilution into account when calcuiatlng the sample concentration.

Expected Values

NT-proBNP concentrations in the following reference groups:.

distributions are 125 pg/mLfor patients younger than 75 years and 450 pg/mL for patients 75 years or older. Each laboratory should establish a reference range that represents the patient population that is to be evaluated.
proBNP Il N-terminal pro B-type natriuretic peptide (3.5 pg/mL), NT-proANP31.67 (preproANP56-e2) (1.0 ng/mL), NT-proANPTe-e6 (preproANPlsa-123) (1.0 ng/ml), renin (50 ng/mL), urodilatin (3.5 pg/ml).

References

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von Beckerath O, et al. Prognostic Value of N-Terminal Pro-Brain
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Ci rculation 2005:112:21 02-21 07.
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Chronic Heart Failure in the Adult: a Report of the American College of
Cardiology/American Heart Association Task Force on Practice Guidelines
(Writing Committee to Update lhe 2001 Guidelines for the Evaluation and
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Available at: http://www. Acc.org/clinical/guidelines/failure/index.pdf
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comparison to the Biosite Triage BNP assay.
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2.2.11 CBC

ARIC Visit 5/NCS Study: Complete Blood Count (CBC) Protocol

Principle:

The ABX Horiba Diagnostics MICROS 60-CS is a fully automated (Microprocessor Controlled) Hematology analyzer. It is used for in-vitro diagnostics testing of whole blood specimens, platelet PRP samples and whole blood component concentrates. The instrument implements both impedance technology and spectrophotometry to determine a Complete Blood Count with 3-part Differential. The 16 parameters are determined with a microsampling of only 10μL. The Micros 60 can analyze approximately 55 samples per hour.

CBC Parameters:

CBC PARAMETERS (16)		
1	**WBC**	white blood cell count
2	**RBC**	red blood cell count
3	**HGB**	hemoglobin
4	**HCT**	hematocrit
5	**MCV**	mean cell volume

6	**MCH**	mean corpuscular hemoglobin
7	**MCHC**	mean corpuscular hemoglobin concentration
8	**RDW**	red cell distribution width
9	**PLT**	platelet count
10	**MPV**	mean platelet volume
11	**LYM %**	lymphocyte percentage
12	**LYM #**	lymphocyte absolute number
13	**MON %**	monocyte percentage
14	**MON #**	monocyte absolute number
15	**GRA %**	granulocyte percentage
16	**GRA #**	granulocyte absolute number

Sample Collection:

Biosafety practices should be followed during both phlebotomy and laboratory handling as specified in OSHA Bloodborne Pathogen Rule (29 CFR Part 1910.1030). Collection of venous whole blood in (1) EDTA 4.5ml vacutainer tube is necessary for this assay. The sample tube must be filled with the exact quantity of blood as indicated on the tube itself. Incorrectly measured blood sample collections will show a variation in results. Invert the sample tube 5-10 times to gently mix the anticoagulant and whole blood before transport. The collection tube should remain at room temperature during both collection and transport. The identification label must include ID number and collection date and time. Samples must be analyzed within 30 hours after collection. Discard the sample if delivery is >30 hours.

Instrument Startup:

Perform a visual check of all reagents. If a reagent level is low, replace the reagent and prime the new bottle. The ABX Micros 60 will run a Startup automatically if the instrument has been idle for more than 4 hours. The Startup cycle will prime all reagents, check electronics and mechanical movements. Then the instrument will perform a Blank cycle for a Background count and print results. If the Background count is above any parameter limits, the ABX Micros 60 will perform another cycle. If the problem persists, refer to the Maintenance and Troubleshooting section of the manual.

Instrument Quality Control:

To monitor the accuracy and precision of the ABX Micros 60 blood cell counter, use ABX MINOTROL 16 (part # 2043756) tri-level control product. MINOTROL is a stable diagnostic reagent composed of human erythrocytes, mammalian leukocytes and platelets in plasma-like fluid with preservatives. Controls are handled in the same manner as a patient specimen. The assay tables for each lot are determined on validated instruments using the appropriate reagents. The tri-level control tubes should remain at 2 to 8^{o}C in an upright position in the original package. DO NOT FREEZE. Unopened tubes are stable until expiration date provided on tube label. Opened tubes are stable for 16 days if handled properly. The reagent technical data sheet should be reviewed when each new lot is delivered (IS421-02 Rev 12/09).

1. Gently remove the control tubes from the refrigerator, inspect for deterioration, the control blood should be similar in appearance to fresh whole blood.
2. Keep tubes upright (DO NOT MIX) and warm to room temperature for 15 minutes on the bench.
3. After 15 minutes, mix tube (Low, #1) by holding horizontally between palms and rolling back and forth for 20 - 30 seconds.
4. Invert tube 8-10 times until the red cell sediment is completely resuspended.
5. Repeat if necessary until all cells are resuspended.
6. Insert QC Smart Card (upper left corner of instrument) with a firm push until it clicks into place.
7. From the QC Menu, select 1 – *AUTOMATIC*.
8. Review Lot information and follow prompts on the screen.
9. Select Operator, use the down arrow to toggle between operators and press ENTER.
10. Press ENTER to select the control level (Low, Normal, High).
11. A message will appear "Press Start to Aspirate"
12. When cycle is complete, the display will indicate: "Close tube holder"
13. Rotate the tube holder to select the appropriate position for the control tube (5ml vacutainers = position 5).
14. Remove the cap from the control tube and wipe threads of the tube and cap with a lint free wipe before closing the tube holder door.
15. After analysis cycle, recap tube immediately.
16. Compare the results with the Hematology Reference Control sheet provided by Horiba for each specific lot. Accept/Reject if parameters are within/outside acceptable limits.
17. To Accept or Reject, press the escape (ESC) key, the prompt will ask 'Valid Low?' choose No: ESC or Yes: ENTER.
18. If you Accept the Low level, the data will be stored on the QC Smart Card and the display will return to SELECT LEVEL (Normal and High)
19. Choose the next control level and repeat steps 14 -18.
20. Gently and thoroughly mix each control tube prior to closing the tube holder door.
21. Repeat for all 3 levels.
22. If the results are outside of the acceptable limits choose Reject and repeat the level until acceptable limits are met.
23. Once the last control level has been accepted, the display will indicate – 'Valid QC' choose No: ESC or Yes: ENTER.
24. Choose ENTER and all levels of controls will be stored on the QC Smart card.
25. Return control tubes to the refrigerator immediately.

Automatic Sampling:

1. After transport, gently and thoroughly mix the EDTA blood collection tube.
2. Place EDTA blood collection tubes on rocker for gentle mixing.
3. Rotate the tube holder to select the appropriate position for the sample tube (5ml vacutainers = position 5).
4. Mix the sample tube by inverting 5 - 10 times prior to placing the tube in the holder.
5. Close the tube holder door.

6. The sample Identification can be entered manually by using alphanumeric characters on the instrument keypad (number pads and up/down arrows) or by pressing the "ID" key and using the bar code scanner.
7. Press the "ENTER" key to save the ID and begin analysis.
8. After analysis cycle, the instrument will automatically create a printout of results.
9. Run a duplicate CBC to compare results for each specimen, record the first data printout if results are within acceptable limits.
10. Results exceeding the linear range or rejected should be re-analyzed.

Reporting Results:

1. Enter each specimen into daily CSV file (format approved by UNC and ARIC Committee)
2. Review each ARIC specimen ID and data point twice before saving the file.
3. Email CSV file or copy to an external drive to save on the ACRL computer (CARDESK4) [password: super5]. (My documents>>ARIC CBC Visit 5)
4. Double click Filezilla Client (desktop-red icon) and connect to UNC (click on the top left green/blue icon or Click>File>>Site Manager), Click CONNECT button.
5. Choose daily CSV file to FTP (file transfer protocol), single click and drag into open space (Right side) 'Empty Directory Listing'
6. Close Filezilla Client.

Standby Cycle:

At the end of the business day, a Standby cycle must be performed. The Standby mode introduces MINICLEAN into the chambers for enzymatic cleaning of protein buildup on the counting chambers and apertures. Press the "Standby" key on the instrument panel to start the cycle. After the Standby cycle is complete, you may leave it in standby mode overnight.

General Maintenance:

1. The instrument exterior can be wiped down with warm water and liquid soap or bleach to remove any dried blood deposits
2. An *Automatic Cleaning* cycle can be activated manually by selecting 4 – Service from the Main menu. Scroll down with the arrow key to select 8 – *Auto Clean*, press enter to activate the cycle.
3. The *Concentrated Cleaning* function performs a very strong cleaning of the WBC and RBC chambers and apertures. Use this function when frequent flags are present on a normal patient analysis or QC is invalid. Select 4 –Service from the Main menu. Scroll down with the arrow key to select 4 – *concentrated cleaning*. Two solutions are recommended: 1) ABX Minoclair or 2) 75% Clorox bleach (3:1, Bleach:DI). Press enter and follow instructions.

Troubleshooting:

Refer to Micros 60 User Manual, section 6 or call Technical Support.

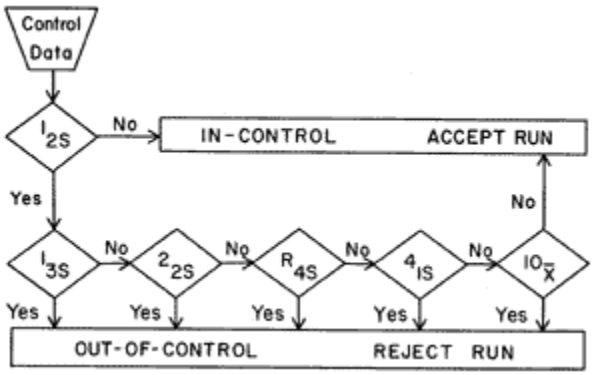
ABX HORIBA Customer Service/Technical Center: 1-888-903-5001
Instrument Serial # 404CS74924
BCM Account # 19789

3. QUALITY CONTROL FOR ARIC ATHEROSCLEROSIS LABORATORY

Definition: Quality control (QC) is a system used to maintain a determined level of accuracy and precision. Proper quality control helps ensure that reported results of patient laboratory testing are correct. Quality control applies not only to specimen testing, but also to collection, storage, and transportation. [1]

The ACRL uses the Westgard Multirule QC rules due to the variety of testing methods performed in the labortory and the number of internal QC pools used. The Multirule QC uses a combination of decision criteria, or control rules, to decide whether an analytical run is in-control or out-of-control. The well-known Westgard multiruleQC procedure uses 5 different control rules to judge the acceptability of an analytical run.

Example: diagram taken from Westgard Quality Management[1]



3.1. Internal Quality Control

Calculations

Control pools are treated the same as unknown samples. The controls are measured in duplicate for statistical purposes. The daily mean, $\bar{x}_1$, for each control pool is defined as the mean of the two aliquots, analyzed in duplicate. The overall mean, $\bar{x}_d$, is calculated by the formula: $\bar{x}_d = \bar{x}_1 / n$, where $\bar{x}_1$ is the daily mean and n is the number of runs.

[1] **Levey-jennings Information and Courses from MediaLab, Inc.**

The overall standard deviation, S, is calculated by the formula: $S = [(\Sigma x^2 - (\Sigma x)^2/n)/n - 1]^{1/2}$ where x is each individual analysis and n is the total number of analyses. Warning limits for the daily mean chart are the following. The upper limit is $\bar{x}_1 + 2S$, while the lower limit is $\bar{x}_1 - 2Sx$. Control limits for the daily mean chart are $\bar{x}_1 + 3$ S for the upper limit and **`x1** – 3 S for the lower limit.

An entire assay will be declared out of control and repeated, when:

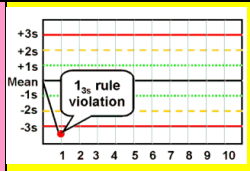
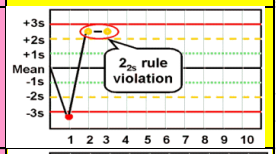
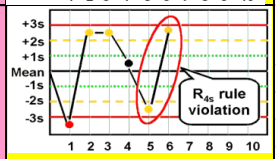
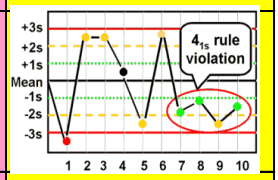
1. $\bar{x}_1$ for two of the three control pools falls outside the control limits.
2. $\bar{x}_1$ two pools fall outside the warning limits on three successive days, or

An assay will be repeated in part, i.e., only on those samples whose apparent concentration content falls in the range of the respective pool, when:

1. $\bar{x}_1$ of the respective pool falls outside the control limits.
2. $\bar{x}_1$ the respective pool fall outside the warning limits on three successive days,

Individual samples will be repeated when the %CV of a given sample exceeds 15% of its mean value. (for assays performed in duplicate-ELISA/EIA/RIA). For automated chemistry and immunochemistry measurements, the acceptable % CV is unique to the analyte.

Quick Quality Control Guidelines Table:

Rule	QC Rules *(Questions to Ask for Ruling)*	In-Control Report Data *(If Answer Is No)*	Out-of-Control, Reject Analytical Run (If Answer Is Yes)	Example *(Graph)*
1_{3s}	Does control data have 1 point outside 3 sigma SD?	No	Yes	
2_{2s}	Does control data have 2 consecutive values outside the same 2 SD across runs or 2 consecutive values outside the same 2 SD within the run?	No	Yes	
R_{4s}	Does control data have a range (difference) between 2 controls within a run exceeding 4 SDs	No	Yes	
4_{1s}	Does control data have 4 consecutive control values on 1 side of the mean & further than 1SD from the mean(within 1 contr across 4 cons. Runs or within 2 controls across 2 cons runs)?	No	Yes	
10_x	Does control data have 10 consecutive value on 1 side of the mean (this can be within 1 contr across 10 cons runs or within 2 controls across 5 cons runs)?	No	Yes	

3.2. External Quality Control

Proficiency Testing (PT):

Evaluations of all proficiency-testing survey reports from the College of American Pathologists are made by the laboratory director(s) or designee and Quality Assurance Team. In the event of a failure, the director is involved with the investigation and resolution.

External quality control is monitored by the following methods:

Auditing systems of The Methodist Hospital and Baylor College of Medicine act as facility regulators. Both the hospital and the college have periodic audits for safety, auditory, environmental, ergonomics, instrument maintenance room temperatures and humidity. These external monitors are essential to quality output.

Use of U.S. Biologicals plasma and serum samples, a CCLSI referenced manufacturer, for source of quality control material when other PT is not available.

Participation in the College of American Pathologist's (CAP) surveys.

The Quality Assurance Program for the Atherosclerosis Clinical Research Laboratory is enforced and is in compliance with the Texas Regulations, and guidelines of Baylor College of Medicine and The Methodist Hospital

Clinical Laboratory Improvement Amendments (CLIA) and College of American Pathologists (CAP) accredit and certify this Laboratory.

Participation in a Blind Study Program Atherosclerosis Risk in Communities (ARIC).

3.3. Data Verification/Rejection:

Chemistry Analyzer

Data generated from the chemistry and immunochemistry analyzers (Beckman Coulter Olympus AU 400 and Roche Cobas e411) are dictated by parameters pre-set to determine values higher or lower than the cut-off used for expected value ranges. The instruments are set to an automatic rerun mode for outside parameter ranges. Data point values are verified by this method but not necessarily rejected. Manual and automated dilutions are made as necessary per instrument.

3.4. Quality Assurance:

The ARIC Central Atherosclerosis Laboratory (ACRL) has a Quality Assurance Plan which includes a Standard Operational Procedures (SOP). This plan incorporates monitors for all laboratory operations. Laboratory restraints are defined by these monitors and they serve as indicators of performance. Strict adherence is mandated. Below is a table listing these monitors.

(**Monitor**) Quality Assurance Plan	(**Monitor**) SOP	(**Monitor**) Quality Control Plan	(**Monitor**) CAP & CLIA Accreditation
(Monitor-Support) QC material from U.S. Biologicals (CCLSI & WHO standards)	(Monitor-Support) Dedicated Backup Server PC	(Monitor-Support) Freezer Monitoring Auto Remote System with Email Notification	(Monitor-Support) Statistical, Freezer Inventory & Database Software
(**Monitor-Support**) Personnel Confidentiality Doc. & Password Protection	(**Monitor-Support**) Participation in Peer-Rated Proficiency Testing	(**Monitor-Support**) 24-Hour Serv. Contracts on all Instruments and testing equipment	(**Monitor-Support**) Monitoring Devices for Room Environment, Tests and Instruments

3.5. References:

1. James O. Westgard, Ph.D., Professor. Department of Pathology and Laboratory Medicine, University of Wisconsin Medical School, Madison, Wicsconsin. “Quality Management.

2. Tietz Textbook of Clinical Chemistry and Modular Diagnostics. Edited by Carl A. Burtis, Edward R. Ashwood and David E. Bruns. Fourth Edition. Elsevier Saunders Inc., Copyright 2006 Chapter 19, “Quality Management” p. 502-523.

3. National Committee for Clinical Laboratory Standards (CCLS): How to define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline, 2nd Edition ed. C28-A2, Vol 20, Number 13. Wayne, PA: National Committee for Clinical Laboratory Standards, 2000.

4. Westgard JO, Barry PL, Quam EF, Shrmeyer SS, Plaut D, Statland BE. Basic QC Practices, Training in Statistical Quality Control for Healthcare Laboratories. 2nd Edition, Westgard QC, Inc. Madison, WI, 2002.

5. **Levy Levey-jennings Information and Courses from MediaLab, Inc., 242 Culver Street, Suite 214, Lawrenceville, GA 30046.**

4. DATA TRANSFER (ACRL)

Data will be transmitted to the Coordinating Center in a cvs string format to be uploaded via the ftp. The string will include the following information:

1. ARIC I.D.
2. Name of test
3. Units
4. Values
5. Date of Run
6. Any Alert value
   - Triglycerides: ≥1000 mg/dL or greater
   - Glucose: ≥ 126 mg/dL
7. Comments

5. LABORTORY METHODS: UNIVERSITY OF MINNESOTA ADVANCED REASEARCH AND DIAGNOSTICS LABORATORY (ARDL) – ARIC CHEMISTRY LABORATORY

5.1. Sample Processing And Storage At The Aric (ARDL) Chemistry Lab

The specimens for University of Minnesota Advanced Research and Diagnostic Laboratory (ARDL) are shipped by Baylor via Federal Express. Typically, packages will arrive on Tuesday mornings.

Processing

1. ARIC processing area located in Mayo, Room D208.

2. Weekly frozen shipments should arrive on Tuesdays in the morning mail (10:00-11:00am).

3. Weekly frozen shipments will arrive in large boxes containing dry ice. Upon arrival, open one shipping box at a time. **Note: Work with one field center's specimens at a time.**

4. The Shipping/Receiving Forms will be in a plastic bag inside the shipping box. Remove the Shipping/Receiving forms. Record the receipt date and time on the Shipping/Receiving Form.

5. Remove the bags of specimens from the shipping box.

6. Each ARIC participant's frozen specimens will be separated according to sample type into five 6 x 6 or 3 x 6 plastic bags, which will be inside a large 11 x 15 plastic bag.

7. Locate the Lab ID on each bag and match the specimens received to the Shipping/Receiving Form. If there are any discrepancies note them on the Shipping/Receiving Form.

8. Place the bags of specimens in a -70 freezer.

9. Take the Shipping/Receiving forms to the computer and enter into the BSI inventory system. Place the vials into the appropriate boxes in the electronic inventory according to the template developed for the ARIC study.

10. Place the Shipping/Receiving Forms in the "ARIC Shipping/Receiving Forms in Progress" folder located in the file cabinet in L275.

11. Place the shipping boxes in the "Box Area" in L275 to be sent back to the field center.

Inventory

1. Line up three Styrofoam covers in a row on the counter.

2. Fill the three large Styrofoam covers with dry ice.

3. Choose one field center to work with; using the Shipping/Receiving Forms as a guide, pull the first field center grouping out of the freezer and place in a dry ice cooler. **Note: The specimens must be placed in the boxes in the same order as the Shipping/Receiving Forms because the specimens were scanned into the inventory using the Shipping/Receiving Forms.**

4. Open the BSI inventory system on the computer. Use the locations function in BSI to match the inventory boxes to ensure the proper specimen positions in the boxes.

5. There are 15 total inventory boxes and 3 working boxes. Place six boxes on each cover of dry ice in the following order:
    a. Serum Box A
    b. Serum Box B
    c. Serum Box C
    d. Serum Box D
    e. Serum Box E
    f. LTS-Primary Serum-Box A
    g. LTS-Backup Serum-Box B
    h. EDTA Box A
    i. EDTA Box B
    j. EDTA Box C
    k. EDTA Box D
    l. LTC-Primary-Cit-Box A
    m. LTC-Backup-Cit-Box B
    n. LTU-Treated to 7.0-Urine-Box A
    o. LTU-Treated to 7.0-Urine-Box A
    p. Serum Chemistry Working Box
    q. Urine Neutral Working Box
    r. Glyhb Working Box

6. Choose the first participant's large bag of specimens (using the order of the Shipping/Receiving Form as a guide). There are 5 small bags of specimens within the large bag.

7. Remove the small bag with the red top vials. This bag should contain 12 vials. Remove the vials from the bag and note if there are missing vials/specimen comments on the Shipping/Receiving Form. **Note: If there are missing vials leave empty spaces in these positions in the box. If needed, place an empty vial in the empty positions to ensure proper vial placement.**

8. Hint: All of the inventory boxes are labeled with the number of vials that should be placed in each box.

9. Place one vial in the Serum Box A.

10. Place one vial in the Serum Box B.

11. Place one vial in the Serum Box C.

12. Place one vial in the Serum Box D.

13. Place one vial in the Serum Box E.

14. Place three vials in the LTS-Primary-Serum-Box A

15. Place three vials in the LTS-Backup-Serum-Box B.

16. Place one vial in the Serum Working Box.

17. Remove the small bag with the purple top vials. This bag should contain 4 vials. Remove the vials from the bag and note if there are missing vials/specimen comments on the Shipping/Receiving Form. **Note: If there are missing vials leave empty spaces in these positions in the box. If needed, place an empty vial in the empty positions to ensure proper vial placement.**

18. Place one vial in the EDTA-Box A.

19. Place one vial in the EDTA-Box B.

20. Place one vial in the EDTA-Box C.

21. Place one vial in the EDTA-Box D.

22. Remove the small bag with the blue top vials. This bag should contain 3 vials. Remove the vials from the bag and note if there are missing vials/specimen comments on the Shipping Form. **Note: If there are missing vials leave empty spaces in these positions in the box. If needed, place an empty vial in the empty positions to ensure proper vial placement.**

23. Place two of the vials in the LTC-Primary-Cit-Box A.

24. Place one vial in the LTC-Backup-Box B.

25. Remove the small bag with the urine vials (1 yellow top and 2 green top vials). Remove the vials from the bag and note if there are missing vials/specimen comments on the Shipping/Receiving Form. **Note: If there are missing vials leave empty spaces in these**

positions in the box. If needed, place an empty vial in the empty positions to ensure proper vial placement.

26. Place the yellow top vial in the Urine Working Box.

27. Place one green top vial in the LTU-Treated to 7.0-Urine Box A.

28. Place one green top vial in the LTU-Treated to 7.0-Urine Box B.

29. Remove the small bag with the black top vial. This bag should contain 1 vial. Remove the vial from the bag and note if there are missing vials/specimen comments on the Shipping/Receiving Form. **Note: If there are missing vials leave empty spaces in these positions in the box. If needed, place an empty vial in the empty positions to ensure proper tube placement.**

30. Place the black top vial in the Glyhb Working Box.

31. Once all the vials from the chosen field center grouping are placed in the boxes, check the BSI inventory system to make sure you have ended on the proper position in each box.

32. Pull out from the freezer another field center grouping and repeat inventory steps #3-#31. Add more dry ice to the Styrofoam covers if necessary during the inventory process.

33. Upon completion of the inventory process, place the Shipping/Receiving Forms in the "ARIC Completed Shipping/Receiving Forms" folder located in the file cabinet in L275. A BSI technologist will enter any specimen comments/missing vials into the BSI system using these forms.

34. Place any remaining dry ice back in the dry ice cooler and put away the Styrofoam covers.

Test Requesting/Labeling Specimens

1. Using the Shipping/Receiving Forms, search in the Order Entry folder of Misys by HMO ID by barcode scanning the ARIC Participant ID number.

2. Follow the instructions for test requesting found on the ARIC Misys Cheat sheet.

3. When routing the test orders remove any duplicate container codes. (2 UR) **Hint: The test codes remaining should be RG, WB, and UR**

4. Write the lab accession number on the Shipping/Receiving Form.

5. Using the MWS function in MISYS, prepare an incomplete laboratory worksheet. This report will be used to check demographics to ensure the tests were ordered correctly in MISYS.
    a. Function: MWS
    b. 1. Build sequence worksheets
    c. 1. Build sequence worksheets manually
    d. Worksheet: FRCH
    e. "Batch number" <enter>
    f. POSITION # : 1 ACC # : Scan all accession number barcodes for the batch
    g. When all accn numbers are scanned, <enter>
    h. <A> to accept the worksheet

i. Note the assigned batch number

6. Print the worksheet you created:
    a. Function: MWS
    b. 2. Print Sequence Worksheets
    c. Printer: 182
    d. 1. Sequence worksheets
    e. 1. Incomplete
    f. INCLUDE COMPLETED SPECIMENS ? (<Y>/N): <enter>
    g. INCLUDE COMPOSED TEXT ? (Y/<N>): <enter>
    h. INCLUDE PRELIMINARY RESULTS ? (Y/<N>): <enter>
    i. INCLUDE CID DATA ? ((A)LL/<C>IDs ONLY/(N)ONE): <enter>
    j. Worksheet: FRCH
    k. Batch number: enter the batch number you noted above.
    l. <A> to accept

7. Check the Demographics on the incomplete FRCH worksheet.

8. Place reviewed FRCH worksheets in the black bin by the Mod P.

9. Correct any demographic errors before proceeding.

10. Open the Serum working box. Locate the Lab ID number (this is part of the patient name; ex: X, J123456) on the MISYS label. Find the vial with same ARIC ID number. Place the small bar-coded Misys CID label for the Roche Mod P serum chemistry tests (URIC and CREATR) on each red vial so the number is perpendicular. Continue this process until all of the red vials in the Serum working box are labeled. **Note: The MISYS labels list the accession number and the Lab ID number for ease in matching the labels to the vials.**

11. Open the Glyhb working box. Locate the Lab ID number (this is part of the patient name; ex: X, J123456) on the MISYS label. Find the vial with same ARIC ID number. Place the small bar-coded Misys CID label for the GLYHB test on each black vial so the number is perpendicular. Continue this process until all of the black vials in the Glyhb working box are labeled. **Note: The MISYS labels list the accession number and the Lab ID number for ease in matching the labels to the vials.**

12. Open the Urine working box. Label an empty 2 mL Sarstadt vial with the small bar-coded Misys CID label for urine tests (CRDUR and UMALBR) on the vial so the number is perpendicular. Continue this process until all of the labels for urine tests are used.

13. Thaw and mix the large 5 mL yellow-capped vial containing urine. Match the ARIC ID number on the vial to the 2 mL Sarstadt vial you labeled with the Misys label (this is part of the patient name; ex: X, J123456). Using a plastic transfer pipette, aliquot 1 mL of urine into the Sarstadt vial. Cap the 2 mL vial using a yellow cap.

14. After all urines aliquots are made, centrifuge the 2 mL urine vials for 10 min at 3100 RPM. Place urine vials back in the working box.

15. Save one large bar-coded urine MISYS label for the ProSpec desk.

16. Save one large bar-coded Glyhb Misys label for the Tosoh desk.

17. Place a large bar-coded MISYS label for each test requested on the participant's Shipping/Receiving Form.

18. Throw away any remaining MISYS labels.

19. Thoroughly mix the serum specimens by inverting the serum working box 10-20 times. Deliver the serum working box(s) to the Mod P desk. The Mod P technologist will place the completed serum working box(s) into the "ARIC Done" container housed in the walk-in refrigerator. The ARIC point person will place the complete serum vials into the Serum Working Box – COMPLETED when al Mod P testing is complete for later Vitamin B12 and TSH testing.

20. Deliver the Urine working box(s) to the Roche Mod P or Pending Mod P box for the urine creatinine testing along with the saved urine Misys labels. The Mod P technologist will deliver the Urine working box(s) to the Prospec desk when urine creatinine testing is finished. The Prospec desk will complete the urine albumin testing and place the finished Urine working box(s) into the "ARIC Done" container housed in the walk-in refrigerator.

21. Thoroughly mix the Glyhb specimens by inverting the Glyhb working box 10-20 times. Deliver the Glyhb working box(s) to the Tosoh desk for Glyhb testing along with the saved Glyhb Misys labels. The Tosoh technologist will place the completed Glyhb working box(s) into the "ARIC Done" container housed in the walk-in refrigerator.

22. All of the specimens in the "ARIC Done" container will be kept for 1 week and then discarded.

23. The working boxes will be re-used.

24. Complete any orders in Misys on samples that were not received or were unacceptable (clotted etc…). Go into <Misys>, <Smar term>, Function: MEM, Worksheet: "put in appropriate worksheet for test" (ex: FRCH, UVR). Type in the accession number that needs a comment entered to complete the order (ex: ;Na-SNR). This step completes the test, otherwise it would continue to be pending**. Note: These tests do NOT need to be credited.**

5.2. The University Of Minnesota Advanced Research And Diagnostics Laboratory (Ardl) Test Methods

5.2.1 Albumin, Urine And Albumin/Creatinine Ratio

Minimal Description For Publication: Albumin is measured in urine using an immunoturbidometric method on the ProSpec nephelometric analyzer (Dade Behring GMBH. Marburg, Germany D-35041).

Principle

A solution of rabbit-derived anti-human albumin is incubated with the urine specimen. An immunocomplex forms between the antibody and the albumin in the specimen, resulting in an increase in light scatter. The higher the concentration of albumin, the more intense the degree of light scatter. The albumin concentration of the test specimen is determined by comparing its light scatter to that observed using known standards in a calibration curve.

Specimen

A random urine specimen not treated with any stabilizer or additive is used for analysis. Specimens are centrifuged for at least 10 minutes at 1,500 x g prior to analysis. This removes are particulate matter that could affect the light scatter measurements.

Equipment

ProSpec nephelometer (Dade Behring GMBH. Marburg, Germany D-35041).

Reagent

Product #OSAL 15. (Dade Behring GMBH. Marburg, Germany D-35041).

Calibration
Dade Behring product #OQIM 13 (3 x 1.0 mL). Albumin concentration will vary with lot. Dade Behring provides periodic calibrator lot and concentration updates on compact disk. When these parameters are read into the system, it is only necessary for the instrument to read the calibrator's barcode to determine its albumin concentration. The reference line is valid until controls demonstrate drift, the reagent lot changes, or the calibrator lot changes. After re-calibration, assay at least five specimens on the old lot and on the new lot. Each of their differences must be within the current posted QC duplicate limit.

Quality Control

There are two levels of controls: one is pooled from routine urinalysis specimens and the other is a dilution of a serum pool. Both controls are assayed with each batch of samples. Consult the quality control detail table for current ranges and pools in use.

In addition to pools, at least one specimen as a within-batch duplicate. The difference in the results must be within the current posted QC duplicate limit.

Expected Values:

Reference range: 0 – 20 mg/g creatinine (see Creatinine method for details)

References:

1. Dade Behring BN ProSpec Nephelometer Instruction Manual. Dade Behring Diagnostics GmbH, Postbox 1149, D35001 Marburg 1, Germany.

5.2.2 Creatinine

Minimal Description For Publication

Creatinine is measured in serum or urine on a Roche Modular P Chemistry Analyzer (Roche Diagnostics Corporation) using a creatinase enzymatic method (Roche Diagnostics, Indianapolis, IN 46250).

Principle:

In this enzymatic method creatinine is converted to creatine under the activity of creatininase. Creatine is then acted upon by creatinase to form sarcosine and urea. Sarcosine oxidase converts sarcosine to glycine

and hydrogen peroxide, and the hydrogen peroxide reacts with a chromophore in the presence of peroxidase to produce a colored product that is measured at 546 nm (secondary wavelength = 700 nm). This is an endpoint reaction that agrees well with recognized HPLC methods, and it has the advantage over Jaffe picric acid-based methods that are susceptible to interferences from non-creatinine chromogens.

Specimen:

Serum from a serum separator tube (biospecimen collection tube #1) or a random urine specimen not treated with any stabilizer or additive is used for analysis. Serum is separated from the cells within 2 hours of collection; both serum and urine are stored at -70° C until assayed.

Interferences:

Bilirubin does not interfere up to an I index of 25. Hemolysis does not interfere up to an H index of 1000. Lipemia does not interfere up to an L index 1000. Cephalosporin antibiotics do not interfere.

Equipment:

Roche Modular P chemistry analyzer (Roche Diagnostics, 9115 Hague Road, Indianapolis, IN 46250).

Reagent: Roche product #1775685, CREA plus reagent kit (Roche Diagnostics, 9115 Hague Road, Indianapolis, IN 46250).

Calibration:

Roche Calibrator for Automated Systems (C.F.A.S.), catalog #759350. The C.F.A.S. calibrator is traceable to reference material SRM 909b (Isotope Dilution Mass Spectroscopy--IDMS). This is a reference material provided by the National Institute of Standards and Technology. This traceability means that this creatinine method yields results that are routinely lower (5-10%) than those creatinine methods using a "traditional" calibrator. The Mod P will automatically perform a two-point calibration when there is a reagent lot number change. It will also perform a two-point calibration every seven days thereafter. The Mod P will not allow testing to proceed until a successful calibration has been completed. Monitor control values to determine stability of the current calibration.

Quality Control:

Two levels of control are assayed each time the ALT method is performed. It is acceptable to run each control at the start of the day, and again at the end of the day. The operator may run them more frequently, if desired. One control is prepared from pooled, normal human serum. The other is an elevated, abnormal commercial control. Consult the quality control detail table for current ranges and lots in use.

Expected Values:

- Reference range, serum: female: 0.4 – 1.1 mg/dL
  male: 0.5 – 1.2 mg/dL

- Linear range of the method: 0-30 mg/dL (serum), 0-600 mg/dL (urine). Specimens exceeding the high limit are automatically diluted (1:2) by the instrument, and reported accordingly. If a manual

dilution is required, dilute the specimen in normal saline, and multiply the result by the dilution factor.

- Analytical Measurement Range: 0-30 mg/dL
- Clinically Reportable Range: 0.1-50 mg/dL

References:

1. Roche/Hitachi System Application Sheet for CREA plus, 2006.
2. Package insert for C.F.A.S., 2005.
3. Roche/Hitachi Modular Analytics Operator's Manual, version 2.0, October 2006.
4. NKDEP Suggestions for Laboratories (Revised December 2005). Internet website: www.nkdep.nih.gov/resources/laboratory_reporting.htm
5. "NKDEP Launches Creatinine Standardization Program", by Richard Pizzi, Clinical Laboratory News, April 2006.
6. "Recommendations for Improving Serum Creatinine Measurement: A Report from the Laboratory Working Group of the National Kidney Disease Education Program", by Gary L. Myers, et. al., Clinical Chemistry, Vol. 52, No. 1, pages 5-18 (2006).

Notes:

1. The MDRD equation for estimating GFR based upon a creatinine value derived from a NIST-traceable calibration is as follows:

Estimated GFR (ml/min/1.73m^2)

$= 175 \times (S_{Cr})^{-1.154} \times (Age)^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if African-American})$

$= \exp[5.228 - (1.154 \times \ln(S_{Cr})) - (0.203 \times \ln(Age)) - (0.299 \text{ if female}) + (0.192 \text{ if African-American})]$

S_{Cr} = serum creatinine in mg/dL
exp = e raised to the power of a given number
ln = natural logarithm of a number

2. Note: The eGFR will be calculated by the HCHS-SOL Coordinating Center using this formula.

5.2.3 D-Dimer

Under development

5.2.4 Glycosylated Hemoglobin

Minimal Description For Publication

Glycosylated hemoglobin is measured in EDTA whole blood using a Tosoh G7 Automated HPLC Analyzer, (Tosoh Bioscience, Inc, South San Francisco, CA 94080).

Principle:

The G7 Automated HPLC Analyzer – HbA1c Variant Analysis Mode uses non-porous ion-exchange high performance liquid chromatography (HPLC) for rapid, accurate, and precise separation of the stable form of HbA1c from other hemoglobin fractions. Analysis is carried out without off-line specimen pretreatment or interference from Schiff base. The analyzer dilutes the whole blood specimen with Hemolysis & Wash Solution, and then injects a small volume of this specimen onto the TSKgel G7 HSi Variant Column. Separation is achieved by utilizing differences in ionic interactions between the cation exchange group on the column resin surface and the hemoglobin components. The hemoglobin fractions (designated as A1a, A1b, F, LA1c+, SA1c, A0, and H-V0, H-V1, H-V2) are subsequently removed from the column by performing a step-wise elution using the varied salt concentrations in the Elution Buffers HSi Variant 1, 2, and 3. The separated hemoglobin components pass through the LED photometer flow cell where the analyzer measures changes in absorbance at 415 nm. The analyzer integrates and reduces the raw data, and then calculates the relative percentages of each hemoglobin fraction. The Total Area of the SA1c is divided by the sum of the total areas of all peaks up to and including the A0 to obtain a raw SA1c percentage. This uncorrected result is substituted as the "x" value in the linear regression formula determined during calibration. The analyzer prints the final numerical results and plots a chromatogram showing changes in absorbance versus retention time for each peak fraction. The Tosoh G7 Automated HPLC Analyzer – HbA1c Variant Analysis Mode is certified by the National Glycohemoglobin Standardization Program (NGSP). The final reportable result is traceable to the Diabetes Control and Complications Trial (DCCT).

Specimen:

Whole blood from EDTA anticoagulated tube (biospecimen collection tube #3).

Interferences: Icterus, as indicated by free and conjugated bilirubin concentrations up to 18.0 and 20.0 mg/dL, respectively, does not interfere with the assay. Lipemia, as indicated by triglyceride concentrations up to 2000 mg/dL, does not interfere with the assay. Concentrations of up to 20 mg/dL of sodium cyanate and acetaldehyde do not interfere with the assay.

Equipment:

Tosoh G7 Automated HPLC Analyzer, (Tosoh Bioscience, Inc, South San Francisco, CA 94080).

Reagent:

DIAMAT HbA1c Sample Preparation Kit, Cat. No. 196-1026 (Bio-Rad Laboratories, Clinical Division, 4000 Alfred Nobel Drive, Hercules, CA 94547).

1. TSKgel G7 HSi Variant Column, Cat. No. 019680 (Tosoh Bioscience, Inc, So. San Francisco, CA 94080).
2. G7 His Variant Elution Buffer 1, Cat. No. 021446 (Tosoh Bioscience, Inc, So. San Francisco, CA 94080).
3. G7 HSi Variant Elution Buffer 1, (S) Cat. No. 019552 (Tosoh Bioscience, Inc, So. San Francisco, CA 94080).
4. G7 Hsi Variant Elution Buffer 2, (S) Cat.No. 019553 (Tosoh Bioscience, Inc, So. San Francisco, CA 94080).
5. G7 Hsi Variant Elution Buffer 3 (S), Cat. No. 019554 (Tosoh Bioscience, Inc, So. San Francisco, CA 94080).
6. Hemolysis & Wash Solution, Cat. No. 018431 (Tosoh Bioscience, Inc, So. San Francisco, CA 94080).

Calibration:

The analyzer has a two-point automatic calibration function. Studies have shown the calibration to be stable for at least seven days. Weekly calibration of the instrument is performed prior to analysis of controls and patient samples. Calibration must also be performed after repeated control failure, major maintenance or service has been performed or whenever a new column is installed.

Quality Control:

Two levels of glycated hemoglobin control (Normal and Elevated) are analyzed in duplicate (or more) with each batch. Controls are prepared from whole blood drawn from a normal (Normal) and a diabetic (Elevated) individual. Stable indefinitely stored at –70°C.

Expected Values:

- Reference Range: 4.3 – 6.0 %
- Linear Range: 3.0 – 19.0 % Results falling outside this range are reported as <3.0 or >19.0 %.
- Clinically Reportable Range: 3.0 – 19.0 % Report results falling outside this range as <3.0 or >19.0 %.
- The American Diabetes Association recommends that a primary goal of therapy should be HbA1c < 7%, and that physicians should reevaluate the treatment regimen in patients with HbA1c values consistently above 8%.

References:

1. G7 Automated HPLC Analyzer Operator's Manual, TOSOH Bioscience, Inc., Inc. 2002.
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4. Nathan DM, Francis TB, Palmer JL. Effect of aspirin on determinations of glycosylated hemoglobin. Clin Chem 1983;29:466-9.
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6. Tze et al. Hemoglobin A1c – An Indicator of Diabetic Control. J of Pediatrics 1978, 93:1316.
7. American Diabetes Association, Standards of medical care for patients with diabetes mellitus (Position Statement). Diabetes Care. 1998;21 (Suppl. 1):S23-S31. G7 Automated HPLC Analyzer
8. Trivelli LA, Ranney HM, Lai H-T. Hemoglobin components in patients with diabetes mellitus. NEJM 1971; 284(7):353.

5.2.5 Plasminogen:

Under Development

5.2.6 Thyroid-Stimulating Hormone (TSH):

Minimal Description For Publication:

TSH is measured in serum using a sandwich immunoassay method on the Roche Elecsys 2010 Analyzer (Roche Diagnostics, Indianapolis, IN 46250).

Principle:

Thyroid-stimulating hormone is measured in serum or plasma on a Roche Elecsys 2010 Analyzer (Roche Diagnostics Corporation) using a sandwich immunoassay method (Roche Diagnostics, Indianapolis, IN 46250). In the first incubation, the patient sample is mixed with a biotinylated monoclonal TSH-specific antibody and a monoclonal TSH-specific antibody labeled with a ruthenium complex to form a sandwich complex. During the second incubation, streptavidin-coated microparticles are added, and the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The microparticles are then captured magnetically, and unbound material is removed. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. The amount of light produced is directly proportional to the amount of TSH in the sample.

Specimen:

Serum collected using standard sampling tubes or tubes containing separating gel and plasma using Li-, Na-, NH -heparin, K3-EDTA, sodium citrate, and sodium fluoride/potassium oxalate as anticoagulant.

Interferences:

The assay is unaffected by icterus (bilirubin < 701 µmol/L or < 41 mg/dL), hemolysis (Hb < 0.621 mmol/L or < 1 g/dL), lipemia (Intralipid < 1500 mg/dL), and biotin < 102 nmol/L or < 25 ng/mL. No interference was observed from rheumatoid factors up to a concentration of 3250 IU/mL and samples from dialysis patients. There is no high-dose hook effect at TSH concentrations up to 1000 µIU/mL. In vitro tests were performed on 26 commonly used pharmaceuticals. No interference with the assay was found. The presence of autoantibodies may induce high molecular weight complexes (macro-TSH) which may cause unexpected high values of TSH. In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

Equipment:

Roche Elecsys 2010 Analyzer (Roche Diagnostics Corporation)

Reagent:

- Streptavidin-coated microparticles (transparent cap), 1 bottle, 12 mL: Streptavidin-coated microparticles 0.72 mg/mL, preservative.
- Anti-TSH-Ab~biotin (gray cap), 1 bottle, 14 mL: Biotinylated monoclonal anti-TSH antibody (mouse) 2.0 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.
- Anti-TSH-Ab~Ru(bpy) (black cap), 1 bottle, 12 mL: Monoclonal anti-TSH antibody (mouse/human) labeled with ruthenium complex 1.2 mg/L; phosphate buffer 100

mmol/L, pH 7.2; preservative.

Calibration

This method has been standardized against the 2nd IRP WHO Reference Standard 80/558. Every Elecsys TSH reagent set has a barcoded label containing the specific information required for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys TSH CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).
Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the specified limits

Quality Control

One commercial control (low range) and a pooled serum control (normal range) are run at the start of the day and then throughout the testing day along with test samples. The range of these controls is established within the laboratory. The values of the controls need to be evaluated as they are run on the instrument. The controls are plotted daily in the spreadsheet 'Elecsys 2010' within the ARDL Q drive, 'Daily QC tally' folder.

Expected Values

Reference Range: 0.4-5.0 mIU/L

The analytical measurement range (AMR) is 0.005 – 750 mIU/L.

The clinical reportable range (CRR) is 0.005 – 750 mIU/L.

Linearity:

0.005-100 µIU/mL (defined by the lower detection limit and the maximum of the master curve). The functional sensitivity is 0.014 µIU/mL. Values below the detection limit are reported by the instrument as < 0.005 µIU/mL. Values above the measuring range are reported by the instrument as > 100 µIU/mL (or up to 1000 µIU/mL for 10-fold diluted samples). The ARDL lab has verified the linearity up to 74.90 mIU/L.

Lower detection limit: 0.005 µIU/mL. The detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Specificity: For the monoclonal antibodies used, the following cross-reactivities were found:
LH 0.038 %, FSH 0.008 %; hGH and hCG no cross-reactivity.

References

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9. Passing H, Bablok W, et al. A General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988;26:783-790.
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5.2.7 Uric Acid

Minimal Description For Publication

Uric acid is measured in serum using an enzymatic colorimetric assay kit and read on the Roche Modular P Chemistry analyzer (Roche Diagnostics, Indianapolis, IN 46250).

Principle

In this method (developed by Town, et. al., and modified by Siedel), uric acid is oxidized by uricase to produce allantoin, CO2 and peroxide. Then the peroxide produced from this reaction is acted upon by peroxidase in the presence of 4-aminophenazone and TOOS (N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline) to produce a red quinoneimine dye end product. It is a two-point, end-point reaction, with measurement occurring at 546 nm (secondary wavelength 700 nm).

Specimen

Use serum or plasma (heparin or EDTA) for the procedure. Other anticoagulants (citrate, oxalate) are unacceptable. Centrifuge the specimen, and remove the serum or plasma within eight hours of collection. Serum or plasma is stable five days at 4ºC, six months at -20°C, and longer at –70ºC.

Interferences:

Bilirubin does not interfere up to an I index of 40. Hemolysis does not interfere up to an H index of 1000. Lipemia does not interfere up to an L index 1000.

Equipment:

Roche Modular P Chemistry analyzer (Roche Diagnostics, 9115 Hague Road, Indianapolis, IN 46250).

Reagent:

Roche product #1875426, Uric Acid Plus reagent kit (Roche Diagnostics, 9115 Hague Road, Indianapolis, IN 46250).

Calibration:

Roche Calibrator for Automated Systems (C.F.A.S.), catalog #759350. The calibrator is stable until the expiration date on the bottle when stored at 4ºC. The lyophilized calibrator is prepared with 3.0 mL of Milli-Q water. Volumetrically add the water, and then dissolve by gentle swirling within 30 minutes. Avoid formation of foam while mixing. The prepared calibrator is stable for eight hours at room temperature, two days at 4ºC, and one month at –20ºC (frozen once). The C.F.A.S. calibrator uric acid setpoint value is traceable to National Institute of Standards and Technology (NIST) reference material SRM 913. Calibration frequency: The Mod P will automatically perform a two-point calibration (saline + C.F.A.S.) when there is a reagent lot number change. The Mod P will not allow testing to proceed until a successful calibration has been completed. Monitor control values to determine stability of the current calibration.

Quality Control:

Two levels of control are assayed each time the uric acid method is performed. It is acceptable to run each control at the start of the day, and again at the end of the day. The operator may run them more frequently, if desired. One control is prepared from pooled, normal human serum. The other is an abnormal commercial control. Consult quality control charts for current ranges and lots in use.

Expected Values:
Reference ranges:

Serum/plasma, male: 3.4-7.0 mg/dL
Serum/plasma, female: 2.4-5.7 mg/dL

- Linear range of the method: 0.2-25.0 mg/dL (serum). Specimens exceeding the high limit are automatically diluted (2:5) by the instrument, and reported accordingly. If a manual dilution is required, dilute the specimen in normal saline, and multiply the result by the dilution factor. Specimens reading below the linear range of the assay should be reported as <0.2 mg/dL.
- Analytical Measurement Range: 0.2-25.0 mg/dL
- Clinically Reportable Range: 0.2-50.0 mg/dL

References:

1. Roche/Hitachi System Application Sheet for Uric Acid Plus, 2005.
2. Package insert for C.F.A.S., 2005.
3. Roche/Hitachi Modular Analytics Operator's Manual, version 2.0, October 2006.

5.2.8 Vitamin B12

Minimal Description For Publication:

Vitamin B12 is measured in serum using a direct chemiluminescent competitive immunoassay method on the Roche Elecsys 2010 Analyzer (Roche Diagnostics, Indianapolis, IN 46250).

Principle:

The sample is first incubated with the vitamin B12 pretreatment 1 and pretreatment 2 during which bound vitamin B12 is released. The pretreated sample is then incubated with the ruthenium labeled intrinsic factor and a vitamin B12-binding protein complex is formed, the amount of which is dependent upon the analyte concentration in the sample. After addition of streptavidin-coated microparticles and vitamin B12 labeled with biotin, the still-vacant sites of the ruthenium labeled intrinsic factor become occupied, with formation of a ruthenium labeled intrinsic factor-vitamin B12 biotin complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

Specimen:

Serum collected using standard sampling tubes or tubes containing separating gel and plasma using Na-heparin and K3-EDTA as anticoagulent. When sodium citrate, sodium fluoride/potassium oxalate are used, the values obtained are by 23 % lower as compared to serum.

Interferences:

The assay is unaffected by icterus (bilirubin < 1112 µmol/L or < 65 mg/dL), hemolysis (Hb < 0.621 mmol/L or < 1.0 g/dL), lipemia (triglycerides < 17.1 mmol/L or < 1500 mg/dL), and biotin < 205 nmol/L or < 50 ng/mL. In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration. No interference was observed from

rheumatoid factors up to a concentration of 1500 IU/mL. In vitro tests were performed on 54 commonly used pharmaceuticals. No interference with the assay was found. In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

Equipment:

Roche Elecsys 2010 Analyzer (Roche Diagnostics Corporation)

Reagent:

- Pretreatment reagent 1 (white cap), 1 bottle, 4 mL: Dithiothreitol 1.028 g/L; stabilizer, pH 5.5.
- Pretreatment reagent 2 (gray cap), 1 bottle, 4 mL: Sodium hydroxide 40 g/L; sodium cyanide 2.205 g/L.
- Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- Intrinsic factor~Ru(bpy) (gray cap), 1 bottle, 10 mL: Ruthenium labeled porcine intrinsic factor 4 µg/L; cobinamide dicyanide 15 µg/L; stabilizer; human serum albumin; phosphate buffer, pH 5.5; preservative.
- Vitamin B12~biotin (black cap), 1 bottle, 8.5 mL: Biotinylated vitamin B12 25 µg/L; biotin 3 µg/L; phosphate buffer, pH 7.0; preservative.

Calibration:

This method has been standardized against the Elecsys Vitamin B12 assay (11820753). Every Elecsys Vitamin B12 reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys Vitamin B12 CalSet II.

Calibration Frequency:

Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).
Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. if quality control findings are outside the specified limits

Quality Control:

One commercial control (low range) and a pooled serum control (normal range) are run at the start of the day and then throughout the testing day along with test samples. The range of these controls is established within the laboratory. The values of the controls need to be evaluated as they are run on the instrument. The controls are plotted daily in the spreadsheet ‘Elecsys 2010’ within the ARDL Q drive, ‘Daily QC tally’ folder.

Expected Values:

Deficient:	32-210 pg/mL
Indeterminate	211-246 pg/mL
Normal	247-911 pg/mL

The analytical measurement range (AMR) is 30.0 – 3,666 pg/mL.

The clinical reportable range (CRR) is 30.0 – 20,000 pg/mL.

Linearity:

22.0-1476 pmol/L or 30.0-2000 pg/mL (defined by the lower detection limit and the maximum of the master curve). Values below the detection limit are reported by the instrument as < 22.0 pmol/L or < 30.0 pg/mL. Values above the measuring range are reported by the instrument as > 1476 pmol/L or > 2000 pg/mL. The ARDL lab had verified the linearity up to 1833 pg/mL.

Sensitivity: Lower detection limit: 22.0 pmol/L or 30.0 pg/mL. The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Specificity: Cross-reactivity of 0.024 % was found with cobinamide dicyanide at 200 ng/mL.

References:

1. Herbert V. Drugs effective in megaloblastic anemias. In Goodman LS and Gilman A (eds): The Pharmacological Basis of Therapeutics, 5th Ed. MacMillan Co. 1975;324-1349.
2. Ross GIM. Vitamin B12 assay in body fluids. Nature 1950;166:270-271.
3. Barakat RM, Ekins P. Assay of vitamin B12 in blood: A simple method. Lancet 1961;2(7192):25-26.
4. Rothenberg SP. Assay of serum vitamin B12 concentration using Co57-B12 and intrinsic factor. Proceedings of the Society for Experimental Biology and Medicine 1961;108:45-48.
5. Rothenberg SP, DaCosta M, Rosenberg BS. A radioassay for serum folate: Use of a two-phase sequential incubation, ligand-binding system. New Eng J Med 1972;285(25):1335-1339.
6. Gutcho S, Mansbach L. Simultaneous radioassay of serum vitamin B12 and folic acid. Clin Chem 1977;23:1609-1614.
7. Kolhouse JF, Kondo H, Allen NC, Podell AB, Allen RH. Cobalamin analogues are present in human plasma and can mask cobalamin deficiency because current radioisotope dilution assays are not specific for true cobalamin. New Eng J Med 1978;299(15):785-792.
8. BIO RAD Quantaphase B-12/Folate Radioassay Instruction Manual, März 1995.
9. Occupational Safety and Health Standards: bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.
10. Council Directive (2000/54/EC). Official Journal of the European Communities No. L262 from Oct. 17, 2000.
11. Passing H, Bablok W, et al. A General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988;26:783-790.
12. Roche Diagnostics. Vitamin B12 immunoassay package insert. Roche Diagnostics 9115 Hague Road Indianapolis, IN 46250-0457.

5.2.9 Von Willebrand Factor Antigen:

Under Development

5.2.10 Cystatin C

Minimal Description For Publication:

Cystatin C is measured in serum on a Roche Modular P Chemistry Analyzer (Roche Diagnostics Corporation) using a turbidometic method (Gentian AS, PO Box 733, N-1509, Moss, Norway).

Principle:

Serum is mixed with cystatin C immunoparticles. Cystatin C present in the sample binds to the antibody bound to the particles, and aggregation occurs. The formed complexes absorb light, and by turbidimetry the absorption is related to cystatin C concentration via interpolation on an established standard calibration curve. Light absorption is measured at 546 nm (secondary wavelength = 700 nm). Rheumatoid factor does not interfere in this assay because the cystatin C antibody is from avian source.

Specimen:

Serum from a serum separator tube (biospecimen collection tube #1). Serum is separated from the cells within 2 hours of collection and stored at -70° C until assayed.

Interferences:

Bilirubin does not interfere up to 80 mg/dL. Hemolysis does not interfere up to 700 mg/dL. Lipemia does not interfere up to a triglyceride level of 1100 mg/dL.

Equipment:

Roche Modular P chemistry analyzer. Roche Diagnostics, 9115 Hague Road, Indianapolis, IN 46250.

Reagents:

Gentian Cystatin C Reagent Kit (Gentian AS, PO Box 733, N-1509, Moss, Norway), reference #1101:

- R1 reagent (1 x 58 mL). MOPS buffered saline, sodium azide. See insert for concentrations. No preparation required. There are approximately 250 tests per bottle.
- R2 reagent (1 x 10 mL). Purified immunoglobulin fraction that is directed against cystatin C. The antibody is covalently bound to uniform polystyrene particles. Human cystatin C was used as the immunogen in the process of generating the immunoparticles. The particle suspension in preserved in a solution of 15 mmol/L sodium azide and antibiotics. No preparation required. There are approximately 250 tests per bottle.
- Storage and stability. Keep reagents stored in refrigerator until use. R1 is stable for 63 days refrigerated on the analyzer. R2 is stable for 63 days refrigerated on the analyzer.

Calibration:

Gentian Cystatin C Calibrator, reference #1012, 1 x 1 mL. The calibrator is stable until the expiration date on the bottle when stored at 4ºC. The calibrator requires no preparation, and is ready for immediate use.
The calibrator is standardized against the international calibrator standard ERM-DA47/IFCC.
Calibration frequency: Perform calibration when the reagent lot number is changed. There are no automatic calibrations based upon time passed for this method. Monitor control values to determine stability of the current calibration.

Quality Control:

Three levels of control are assayed each time the cystatin C method is performed. It is acceptable to run each control at the start of the day, and again at the end of the day. The operator may run them more frequently, if desired. One control is prepared from pooled, normal human serum. The others are commercial controls of varying concentrations. Consult quality control charts for current ranges and lots in use.
In January 2012 this method, calibrated with the Gentian calibrator, was evaluated by assaying the ERM-DA47/IFCC reference material. This standard has an assigned value of 5.48 mg/L. Eight replicates assayed over four days yielded an average value of 5.62 mg/L. This reference material should be measured periodically to assure method accuracy.

Expected Values:

- Reference ranges:
  Serum, adult: 0.51-1.05 mg/L
  Serum, 5-15 years: 0.51-1.05 mg/L
- Linear range of the method: 0.32-8.00 mg/L (serum). Specimens exceeding the high limit are automatically diluted (2:3) by the instrument, and reported accordingly. If a manual dilution is required, dilute the specimen in normal saline, and multiply the result by the dilution factor.
- Analytical Measurement Range: 0.32-8.00 mg/L
- Clinically Reportable Range: 0.32-20.00 mg/L

References:

1. Package insert for Gentian Cystatin C Reagent Kit, June 2011.
2. Package insert for Gentian Cystatin C Calibrator, May 2011.
3. Roche/Hitachi Modular Analytics Operator's Manual, version 2.0, October 2006.

6. QUALITY CONTROL (QC) AND QUALITY ASSURANCE (QA) – UMN (ARDL)

6.1. Quality Control Calculations

Internal quality control procedures monitor analytical performance relative to medical goals and alert analysts to unsatisfactory analytical performance. Quality control statistics are used to make judgments about the quality of analytical results, whether system correction is necessary, whether

patient data should be accepted or rejected, and for estimating performance parameters which can be compared to analytical and medical goals.

New Method or Instrumentation

Calculate a new permanent SDo and duplicate range whenever a new method or instrument is put into use.

1. Calculate mean, SDo, and coefficient of variation (CV).
   a. Analyze 10-50 control values on 10-50 different days. (Values obtained on different analyzers on the same day may be considered to be "different days".) Use > 20 values for procedures not performed daily (at least 20 values); use > 30 values for procedures using highly automated systems; use > 10 values for procedures performed less than weekly.
   b. Within each day, randomly select the control value used to establish the ranges. Do not exclude a value unless the current control is unacceptable.
   c. Inspect data for outliers; do not exclude a value from the data unless outside the 3SDo limits.
   d. When possible record control values to one more significant digit than reported patient values. Calculate significant digits for QC limits using the following guidelines:

	Preferred Method	Instrument reports QC and patients to same number of digits:
Report patients	XX.	XX.
Measure control	XX.X	XX.
Calculate control mean	XX.XX, round to XX.X	XX.X
Calculate SD	X.XX, round to X.X	X.X
Calculate ranges	XX.X ± X.X	XX.X ± X.X
Design control charts	Plot mean XX.X; plot SD limits to XX.X	Plot mean XX.X; round SD limits to XX

Instruments that chart quality control data may require some exceptions.

2. To calculate the duplicate range for patient samples, refer to the "Duplicate Range" section; additional data may be needed.
3. Document on the Cumulative Control Tabulation Sheet. Submit to the technical supervisor or designee for approval.

New Lot of Quality Control

1. Calculate a new mean using 20 control values analyzed on 20 different days. (Values obtained on different analyzers on the same day may be considered to be "different days".) If necessary, establish a temporary mean using fewer than 20 values. Recalculate when 20 values are available. For procedures performed less than weekly use a minimum of 10 values. Apply permanent SDo and CV to establish new confidence limits.
2. Calculate SDo and CV using 20 control values to monitor the permanent SDo and CV. If new permanent ranges (confidence limits) need to be established, additional values must be collected (see previous section).
3. Document calculations on the Cumulative Control Tabulation Sheet. Submit to the technical supervisor or designee for approval.
4. For unassayed controls, the laboratory must establish a valid acceptable range by repetitive analysis in runs that include previously tested control material. For assayed controls, the laboratory must verify the acceptability ranges supplied by the manufacturer.
5. Purchased calibrators are not to be used as controls.

6. If calibration and control materials are not commercially available, the calibrator or control should be assayed in duplicate for 20 consecutive days to establish a new range. This is required to verify the accuracy of patient/client test results.

Quality Control Statistics

1. When possible, use control tabulation and charting capabilities of automated instrumentation or the Laboratory Information System (LIS). When automated control charts are not available, design manually tabulated control charts to include mean, 2SDo and 3SDo confidence limits. Scale the y-axis to provide a concentration range from mean minus 4SDo to mean plus 4SDo.
2. Use three Westgard rules to determine whether data is acceptable (i.e., in statistical control) to be reported.

RULES	**BATCHED TESTING**	**NON-BATCHED TESTING** (continuously reported testing):
Warning Rule: 1-2s	If one control exceeds the 2 SDo limits, apply rules below before accepting or rejecting the analytical run.	
Action Rule: 1-3s	Reject the run if one control exceeds the 3SDo limits.	If one control exceeds the 3SDo limits: • Reanalyze the control • Check lot number in use, e.g. slide, reagent, control material • Check calibration for slide/reagent lot in use • Analyze performance verifiers if available • Check patient duplicates • Consult the Charge CLS • Perform result correction as necessary.
Action rule: 2-2s	Reject the run if two consecutive controls exceed the same 2SDo limit (mean plus 2SDo or mean minus 2SDo). (This rule applies to two levels in the same run or two consecutive controls of the same level in two consecutive runs.)	1) If two levels of control (analyzed simultaneously or consecutively) exceed the same 2SDo limit (mean plus 2SDo or mean minus 2SDo), or 2) If one level of control exceeds the same 2SDo limit (mean plus 2SDo or mean minus 2SDo) two consecutive times: • Check to see if there is an explanation; if so, continue analyzing and reporting patient samples. • If there is no explanation, repeat the control. If the control still exceeds the 2SDo limit, consult the Charge CLS.
Accept/ Reject:	Accept the run if the rules indicate that the run is in statistical control.	
Any exception in reporting results from the above requires the approval of the Charge Technologist or Technical Supervisor.		

3. Document action taken when control values are unacceptable (e.g. reagent change) and other pertinent information on the control chart. For automated systems, this information may be recorded on a tally and transferred or filed with the monthly printout of the control chart.

4. The responsible CLT/CLS plots and initials the manual quality control each day the test is performed. Record the initials of the person performing the analysis if known; otherwise, record the initials of the individual plotting the data. In LIS, the responsible person is identified by their

LIS logon which is attached to the entry.

5. The Technical Supervisor or designee reviews and initials quality control records monthly and compares imprecision statistics (CV or SD) to previous data. If a significant change is noted, i.e., two times previous CV or SD, investigate the cause, e.g., trending, lot changes, service calls, calibration, or other contributing factors.

Duplicate Range

Calculate the statistical confidence limits for duplicates (used to check precision) by one of several methods. For each analyte determine whether the within day duplicate range, the between day duplicate range, or both will be utilized. In all cases it is important that the appropriate within or between batch SD, CV, or $\bar{R}$ for duplicates be used. It is not possible to predict how within and between batch SD (or CV) relate to each other.

	WITHIN DAY DUPLICATES	**BETWEEN DAY DUPLICATES**
WHEN TO USE:	To evaluate duplicate determinations within an analytical run.	To evaluate duplicate determinations between days and in combination with intra-individual biological variability information, to help determine whether a change in a patient value is statistically significant.
METHOD 1	Use the SDw for the control material.	Use the SDo for the control material.
METHOD 2	Use the CV for the control material calculated from an SDw.	Use the CV for the control material calculated from an SDo.
METHOD 3	Use the $\bar{R}$ from a series of 20 or more control material within day duplicates analyzed over a 1-20 day period.	N/A
METHOD 4	Use the $\bar{R}$ from a series of 50 or more patient within day duplicates analyzed over a 1-50 day period.	Use the $\bar{R}$ from a series of 50 or more patient duplicates analyzed between day.
NOTE: Theoretically, all four methods should give identical answers if: 1) the control materials behave identically to patient samples, and 2) the SD is independent of analyte concentration. However, these assumptions are often not correct. To determine whether control materials behave similarly to patient samples, compare the duplicate range calculated by method 1, 2, or 3 with that calculated by method 4. For some assays, neither SD nor CV are constant over the range of analyte concentrations, and it may be difficult to obtain patient samples for duplicate determinations over the potential range of patient values. In these instances, use the SD for the control material at various levels to calculate duplicate ranges using method 1. (This method assumes control materials behave like patient specimens.)		

Select the correct method(s) to calculate appropriate duplicate ranges (95% confidence limits).

1. If the SD is constant over the entire range of analyte concentrations and patient specimens behave like the control material:

$Duplicate_W$ range (absolute value) = 2.77 x SD_W
$Duplicate_O$ range (absolute value) = 2.77 x SD_O

Calculate SD_W using 10-25 control values analyzed the same day.

2. If the CV is constant over the entire range of analyte concentrations and patient specimens behave like the control material:

   $Duplicate_W$ range (% value) = 2.77 x CV_W
   $Duplicate_O$ range (% value) = 2.77 x CV_O

3. If patient specimens behave like the control material and a within day duplicate range is needed (but an SDw is not available), use the $\bar{R}$ of 20 or more control material duplicates:

   $Duplicate_W$ range (absolute value) = 2.46 x $\bar{R}_W$

4. When quality control materials behave significantly differently from patient samples, for example with blood gas analysis, use the $\bar{R}$ of 50 or more patient specimen duplicates representing the reportable range:

   $Duplicate_W$ range (absolute value) = 2.46 x $\bar{R}_W$
   $Duplicate_O$ range (absolute value) = 2.46 x $\bar{R}_O$

   Plot the absolute difference between duplicate determinations vs. the average of the two duplicate values to determine whether the same duplicate range can be applied to patient values over the entire instrument analytical range. If the duplicate range appears to be independent of analyte concentration, the duplicate range can be applied at all concentrations.

   If the reproducibility of duplicates seems to change as analyte concentration changes, estimate the duplicate range for two or more ranges of patient values by collecting 50 or more duplicates within each range of values or decision points to determine an individual $\bar{R}$ for each range.

   Use of this method to approximate duplicate range at a concentration very different from that of the 50 duplicates is not recommended, because: 1) the formula used in method 4 assumes the SD is constant over analyte concentrations, and 2) for many assays (e.g., most immunoassays) there is no reason to expect the CV to remain constant over the entire range of analyte concentrations.

   Alternatively, estimate the duplicate range at one concentration from the duplicate ranges at another concentration by calculating the duplicate range as a percentage, rather than absolute value. Fundamentally, this approximation assumes the CV, rather than the SD, is constant over the analytical range of the instrument.

   Duplicate ranges for multiple instruments, e.g., sodium on the Vitros 950 5,1 vs. Vitros 350 vs. Radiometer 725/825, is based on the $\bar{R}$ for patient specimens. The $\bar{R}$ between different analyzers is calculated and the largest $\bar{R}$ is used to calculate the duplicate range between all analyzers.

Dilution Guidelines

Definitions:

- The analytical measurement range (AMR) is the range of analyte values that a method can directly measure without any dilution, concentration, or other pretreatment not part of the usual assay process.
- The clinically reportable range (CRR) is the range of analyte values that a method can report as a quantitative result allowing for specimen dilution, concentration, or other pretreatment used to extend the AMR. For example, if it is desired to report a result that exceeds the AMR, the specimen is commonly diluted to bring the analyte into that range; the diluted specimen is reassayed, and the final result calculated using the dilution factor.

If the method CRR exceeds AMR, specify protocol for dilution:

- If dilution is appropriate, specify in the individual procedure diluent and dilution protocol, e.g., keep dilution to a minimum. If not specified, dilution factors are based on the instrument printout or previous patient result and specimens diluted until the result falls within the AMR.
- If dilution is appropriate, specify calculation protocol, e.g., automated dilution and calculation by instrument, manual dilution and instrument calculation, or manual dilution and calculation.
- If dilution is not appropriate because results are reported as greater than a specified value, indicate not to dilute and include appropriate reporting protocol in the individual procedure.

6.2. Quality Assurance Systems

Reference Standards

Analyze aqueous standards or protein-based calibrators with all analytical runs whenever appropriate. Check permanent calibrations at least every six months.

Where applicable, use NIST standard reference material to prepare the standard or to check the material used as the standard. Prepare stock standards at least yearly. Check new stock standards against current stock standards to a stated tolerance, usually $\pm$1% of the nominal value, before introduction into use. Dilute working standards from a stock standard which has been checked. Check working standards according to the requirements of the method, most commonly by assaying against the current standards to ensure they read within marker range, as defined in the individual analytical procedure.

Controls

Analyze two or more levels of controls daily, whenever possible. Evaluate quality control using ranges established in the ARDL laboratory or manufacturers' stated ranges.

The following control materials may be used:

1. Commercial liquid bovine or human based serum
2. Commercial lyophilized bovine or human based serum control
3. Commercial lyophilized urine control
4. Frozen human donor pools (tested to be negative for HIV and hepatitis B and hepatitis C) prepared by the laboratory

External Proficiency Surveys

The ARDL Laboratory participates in a number of proficiency testing surveys provided by such organizations as CAP and CDC. The reports submitted are signed by the staff performing the assays and Laboratory Manager when appropriate. Deficiencies are documented.

Reporting of Results

Define the lowest and highest concentration for each analyte which is reported, based on the linearity, sensitivity, precision and clinical utility of the method.

Define technical limits in the laboratory computer which represent "impossible" values, whenever possible.

Report results to no more than three significant figures, e.g., report 1286 U/L as 1290 U/L or pH 7.386 as 7.39. An exception to this policy is instruments which are on-line to the Laboratory computer.

Quality Assurance Systems in Operation to Detect Errors or Unusual Laboratory Results

In order to minimize the possibility of clerical and analytical errors, the ARDL Laboratory utilizes a laboratory computer system for the entry and verification of test results.

1. Review all results against raw instrument data to ensure the proper calculation and interpretation of results.
2. Perform result entry via computer interface for instruments with high volume tests to avoid errors in manual entry.
3. After computer entry and prior to reporting results, review results on the CRT or computer-generated printout against the original protocol book. Whenever possible, review results against the patient's previous result (delta check value) to detect possible discrepancies.
4. Analyze daily or periodic duplicate specimens to check the analytical performance in laboratory areas where duplicate instrumentation performs the same analytical tests. Take corrective action if necessary.
5. Refer unusual or questionable laboratory results to the supervisor. If appropriate, the supervisor will refer to a faculty member or Laboratory Medicine resident via an Action Report.
6. Consult with supervisor about remedial action to be taken when calibration or controls fail to meet criteria for acceptability.
7. Call results which exceed critical limits to immediate attention per specific study protocol.
8. Use backup equipment or consult a supervisor if a test system becomes inoperable.

General Quality Assurance Systems

1. Record temperature and humidity as necessary.
2. Reagent labels must include storage requirements, name of reagent, concentration, date prepared, date received, date of expiration, and special safety information. Purchased prepared reagents must be labeled appropriately.
3. New or revised methods must be validated before being put into use. New or revised test report information from LIS must be checked before use.
4. All procedures must be reviewed annually.
5. Complete Incident Reports when appropriate.
6. Document complaints, problems and other feedback on "Customer Feedback Log" located near the telephones. These items are reviewed by Laboratory Manager, and appropriate follow-up is initiated.

6.3. References:

1. Youden, WJ. Statistical methods for chemists. New York: John Wiley & Sons, 1951: 8-23.
2. Natrella, MG. Experimental statistics. Washington, D.C.: U.S. Government Printing Office, 1963: 2-6, 2-7, T-18, T-19.

3. Westgard JO, Barry PL, Hunt MR. A multi-rule shewhard chart for quality control in clinical chemistry. Clin Chem 1981; 27:493-501.
4. Westgard, JO, Quam EF, Barry PL. Selection grids for planning quality control procedures. Clin Lab Science 1990; 3:271-8.
5. National Committee for Clinical Laboratory Standards. Internal quality control: Principles and definitions; Approved Guidelines, NCCLS document C24-1 (ISBN 1-56238-112-1). NCCLS, 771 East Lancaster Avenue, Villanova, PA 19085, 1991.

7. THE UNIVERSITY OF TEXAS GENETICS LABORATORY PROTOCOLS AND DNA SAMPLE STORAGE – ARIC GENETICS LABORATORY

7.1. DNA Isolation Using Puregene®DNAPurification Kit For ARIC Contract Buffy Coat Samples

Purpose

1.0 The purpose of this document is to describe the protocol for the extraction of genomic DNA from buffy coat samples using the Puregene® DNA Purification Kit.

2.0 The purpose of this protocol is to isolate genomic DNA from a buffy coat (white blood cell layer of whole blood). The method primarily uses the Puregene® protocol and accompanying reagents as outlined in the Gentra® Puregene® Handbook_Third Edition_April 2010, but some modifications were made to increase the quality and quantity of DNA.

Referenced Documents

- Puregene® DNA Purification Kit
- Gentra® Puregene® Handbook_Third Edition_April 2010
- Qiagen development team/technical support staff information in response to specific questions.

Forms and Attachments

Required Equipment and Components:

Equipment/Components	Vendor	Part number
Eppendorf (micro)centrifuge 5417C	Thermo Fisher Scientific	05-406-11
Sorvall Legend RT+ centrifuge	Thermo Fisher Scientific	75004377
Model RF 1555 Incubator	VWR Scientific	9120982
50 mL conical tubes (Falcon brand)	Thermo Fisher Scientific	35 2098
50 mL conical tubes (Crystalgen)	Phenix	SS-2262
RBC Lysis Solution	Qiagen	158904
Cell Lysis Solution	Qiagen	158908

Protein Precipitation Solution	Qiagen	158912
DNA Hydration Solution	Qiagen	158916
Transfer pipets	Thermo Fisher Scientific	13-711-7M
Eppendorf microtubes (1.7 mL)	ISC Bioexpress	C-3230-1
Attached screwcap microtubes (2.0 mL)	Phenix	SC-332-6S
Ethyl alcohol (200 proof)	Pharmco Aaper	E200GP
Isopropyl alcohol (99.9%)	Thermo Fisher Scientific	BP2632-4
Pasteur pipets (5-3/4 in.)	Thermo Fisher Scientific	3-678-6A
Nuclease free water	Thermo Fisher/Promega	P1195

2. Safety Considerations

Overview

- Wearing of appropriate lab clothing (no open toed shoes) including lab coat.
- Handling blood samples with caution by use of gloves, mask and/or protective eyewear for samples which may be labeled as hazardous due to compromised status of patients involved in study.
- Proper disposal of materials used with blood samples by use of hazardous waste disposal bags that are later autoclaved, special bagged and left for housekeeping to dispose of in waste dumpsters on site.

Procedure

Overview

- Preparation of labware for use with buffy coat handling.
- Preparation of genomic DNA isolation and Puregene® reagents.
- Preparation of frozen buffy coat samples for genomic DNA isolation.
- Day 1: Cell lysis steps during buffy coat sample handling.
- Day 2: Protein precipitation of samples from Day 1 prep.

DNA Precipitation.

- Clean up of DNA isolation materials; handling of waste materials.
- Optional Re-precipitation protocol.

DNA Sample Storage.

Notes on DNA isolation protocol.

Preparation of labware for use with buffy coat handling:

- Label the appropriate number of 50 mL conical tubes (25/pack) and label with pre-printed barcode labels on side. Label the top and side with the sequential "order#" label. Label the Styrofoam tube holder with tape on the front and side indicating the range (by order#) of the set.
- Obtain a set of 2.0 mL attached screw top tubes. The label with "original gDNA" should be placed on the 2.0 mL tube and placed in an aliquot box. Label the aliquot box indicating the range (by order#) of the set.
- Prepare the closed-end glass pipettes by placing the tips of Pasteur pipettes over a Bunsen burner for a few seconds to "seal" the open end.

Preparation of genomic DNA isolation and Puregene® reagents:

- Prepare a liter solution of **70% ethanol** by adding 700 mL of 100% ethanol (200 proof stock) to 300 mL of H2O (cell culture grade; Milli-Q filtered).
- Prepare a liter solution of 0.1X TE buffer by adding 100 mL **DNA Hydration Solution** (1X TE) to 900 mL of H2O (nuclease-free).

Puregene® reagents: RBC Lysis Solution, Cell Lysis Solution, Protein Precipitation Solution, and diluted DNA Hydration Solution will be used as later described in the sample isolation sections**.**

Preparation of frozen buffy coat samples for genomic DNA isolation:

Frozen buffy coat samples should be thawed quickly in 37°C incubator (15-20 minutes) and stored on ice until prepared for lysis steps of the protocol.

Day 1: Cell lysis steps during buffy coat sample handling:

- Thaw batches of 24 buffy coat samples at a time as previously described and continue to next step.
- Add the entire buffy coat preparation to a labeled 50 mL conical tube (Crystalgen brand with dark blue cap). If there is less than 1 mL of buffy coat, identify the 50 mL tube with a red "X" on the top and side of the tube. **NOTE:** you will need to adjust the reagent volumes accordingly in the following procedure to accommodate any of these low buffy coat starting volumes.
- Add 1.5 mL of **RBC Lysis Solution** to the sample with residual buffy coat, mix and add to 50 mL conical tube. Add **RBC Lysis Solution** to the 50 mL conical tube to bring the total volume of the tube up to 40 mL. Cap tube and invert to mix (at least 10 times). Incubate for 10 minutes at room temperature, inverting at least once during the incubation period. Repeat for additional 23 samples.
- Centrifuge the 24 pack of samples for 17 minutes at 2,800 rcf at 25°C.

- Aspirate the supernatant, leaving behind the white cell pellet and a small amount of liquid (10-20 uL). Repeat steps 2 to 4 if necessary.

The white cell pellet can be dislodged and resuspended by: a) tapping the tube on the counter top until the pellet is mixed with the liquid in the tube, or b) rasping/raking the tube 3-5 times across a tube rack to dislodge the pellet and mix with the liquid. **NOTE:** the method of handling for this step is determined by the sensitivity of the samples as older samples will need the more gentle/less vigorous method of handling before proceeding to the next step.

Add the following amounts of **Cell Lysis Solution** based on the initial amount of buffy coat available: add 8 mL of **Cell Lysis Solution** if original buffy coat was 1.0 – 1.5 mL; add 6 mL of **Cell Lysis Solution** if original buffy coat was <1.0 mL (tube with red "X").

Check that the caps are sealed tightly and mix by inverting the tubes several times. Incubate the samples overnight at 37°C by placing on a "rocker" to keep them agitated during incubation. Repeat steps 1-8 for sample batches of 24 or 25 at a time.

Day 2: Protein precipitation of samples from Day 1 prep:

Remove the samples that incubated overnight at 37°C and mix by inverting the tubes several times. Allow to cool to room temperature for 20 to 30 minutes. Process samples in batches of 24 or 25. **NOTE:** Samples at this stage are stable at room temperature for up to 2 years.

Add the following amounts of **Protein Precipitation Solution (PPS)** based on the amount used and/or color of **Cell Lysis Solution:** a) Add 3.1 mL **PPS** to samples with 8 mL **Cell Lysis Solution** or dark red in color; b) Add 2.5 mL **PPS** to samples with 6 mL **Cell Lysis Solution** that are light red in color; c) Add 2.2 mL **PPS** to samples with 6 mL **Cell Lysis Solution** or very light red in color.

Vortex samples gently (low speed) until solution is homogenous.

Centrifuge samples at 2,400xg for 12 minutes at 25°C. The precipitated proteins will form a tight white pellet. If the pellet is not tight, repeat steps 3 and 4.

DNA Precipitation:

Pour the supernatant containing the DNA (being careful to leave behind the precipitated protein pellet) into a labeled 50 mL conical tube containing 9 mL **100% Isopropanol** (2-propanol). **VERIFY THE ARIC ID **BEFORE** POURING THE SUPERNATANT.

Place samples on rocker platform and rock until DNA precipitates.

Verify that the ARIC ID on the 50 mL conical tube matches the 2.0 mL tube about to be used. Do not use the SID for confirmation.

Spool off the DNA with a closed-end glass pipette. Wash the DNA in 500 uL of 70% ethanol in your 1.7 mL tube for about 10 seconds. Then allow the DNA on your pipette end to air dry for about 30 seconds. Add the DNA to the 2 mL screw top tube containing 500-700 uL of 0.1X TE buffer

(diluted 1X **DNA Hydration Solution**), mixing to ensure that the DNA leaves the pipette end and begins to dissolve in the TE buffer.

Re-cap the 50 mL conical tube. Discard the used, glass pipette into the sharps container.

Optional (if spooled DNA is not okay):

- If DNA is fragmented and cannot be spooled, set aside the 100% isopropanol tube in the "problem" sample rack. Make note in the DNA extraction log and log out after use.
- If DNA is fragmented, but can be spooled, or contains heme (orange/red color), set aside the 500-700 uL of 0.1X TE buffer in the "problem" sample rack. Make note in the DNA Extraction log and log out after use.

Incubate the screw cap tubes (containing the DNA) for a **minimum of 2 days** at 37°C on the rotator for holding small tubes.

Update the DNA Extraction log. Log out after use.

Clean up of DNA isolation materials; handling of waste materials:

Waste containers for the used ethanol and isopropanol are available in room W424. Used ethanol can be discarded the same day and empty tubes placed in orange biohazard bags.

Isopropanol tubes must be checked for leftover DNA by a second individual. If the DNA extraction log has been completed, the isopropanol can be discarded in the waste container for ethanol/isopropanol disposal and the tubes can be placed in the orange biohazard bags. If the DNA Extraction log has not been completed, the isopropanol can be discarded, but save the tube until it has been verified as completed.

Waste material in the orange biohazard bags needs to be autoclaved in the basement in Room B01A. The autoclaved bags are then placed in the barrel in the same room which contains a black liner. Housekeeping will dispose of these bags in the evening.

Optional Re-precipitation Protocol:

Place the 24-pack of 50 mL conical tubes with isopropanol mixture in the centrifuge for 8 minutes at 3000 rpm (2000xg) at 25°C. Any remaining DNA will be at the bottom of the tube.

Carefully aspirate off the supernatant, leaving a small amount of liquid at the bottom of the tube. Add 10 mL **70%** ethanol and invert the tubes several times to wash the DNA pellet.

Centrifuge at 3000 rpm for 8 minutes. The DNA pellet should be visible. Carefully aspirate off the ethanol.

Invert and drain the tube on clean absorbent paper and allow sample to air dry approximately 5 minutes.

Add 300 uL of 0.1X TE buffer to pellet. Resuspend and place in pre-labeled 2 mL tubes.

Incubate the screw cap tubes (containing the DNA) for a **minimum of 2 days** at 37°C on the rotator.

7.2. DNA Sample Storage:

Store samples at 4°C. For long-term storage, store at -20°C or -80°C.

Notes on DNA isolation protocol:

- The DNA isolation preparation for the ARIC Visit 1, 2, 3, 4, and 5 buffy coat samples is based on ~1.5 mL buffy coat sample which was prepared from 10 mL of whole blood sample. The expected yield range=200-400 ug DNA.
- The preferred method of obtaining DNA from the precipitation step is spooling as it assures a cleaner DNA sample and eliminates the addition of excess proteins to the DNA pellet if the sample is centrifuged instead (see re-precipitation step).
- Technical notes for use of Puregene® reagents indicate that "when processing buffy coats, the prep should directly scale the volume of reagents used in proportion to the volume of the original blood sample (e.g., if a 1 mL buffy coat sample was prepared from 10 mL whole blood, use the volumes of reagents given for 10 mL of blood."

7.3. Cell Cryopreservation Procedure

Overview

The purpose of the following protocol is to cryopreserve cells for later transformation. The CPT tubes were shipped via overnight delivery from the four ARIC field centers to the ARIC Atherosclerosis laboratory for daily processing. Samples will be couriered from the ARIC Atherosclerosis Laboratory to the ARIC Genetic laboratory.

Supplies Needed

Product	**Vendor**	**Part #**
PBS (1x)	Invitrogen	20012-027
Cryobox 1.2/2.0 mL (9x9)	ThermoFisher Scientific	15-350-107B
2.0 mL Nalgene 5000-0020	ThermoFisher Scientific	03-337-7D
Recovery Cell Culture Freezing Medium (50 mL)	Invitrogen	12648-010
15 mL conical tubes (Falcon brand)	Phenix	SS-2265
Pipettes - 25 ml serological	ThermoFisher Scientific	13-668-2
Pipettes - 10.0ml serological	ThermoFisher Scientific	13-675-20
Transfer pipets (sterile; individ. wrap)	ThermoFisher Scientific	13-711-20
2.0 mL attached screwcap microtubes	Phenix	SC-332-6S
Kimwipes, 15"x17"	ThermoFisher Scientific	06-666C
Labels, 1.0"x1.0"	Phenix	THT-179-492-3
Black Ribbon	Phenix	R-6406
Label, T Spots.(20 sheets)	Diversified Biotech	SPOTS-1000
Label, 2 mL size	ISC Bioexpress	L-1000-9
Ethyl alcohol (200 proof)	Pharmco Aaper	E200GP

Isopropyl alcohol (99.9%, 4L amber glass)	Thermo Fisher Scientific	BP2632-4
Nuclease free water (150 mL)	ThermoFisher/Promega	PR P1195
Pharmatex (Med)	Phenix	GEL-155M
Liquid Nitrogen	Matheson Tri-Gas	NI-160LLP
Lab coats disposable, white XL	VWR	10845-018
C-Fold Paper Towels	ThermoFisher Scientific	19-120=2484
HB Quat Disinfectant Cleaner	ThermoFisher Scientific	14-415-19
Biocision “cool cell” (BCS-136)	ThermoFisher Scientific	13-900-635
Contamination Control Mats	ThermoFisher Scientific	19-166-855

7.3.1. Protocol For Cell Cryopreservation

Laboratory procedure for regular collection (within 24 hrs.) consists of the following steps:

1. The FedEx overnight shipment will arrive at the ARIC Atherosclerosis Laboratory at the Methodist Hospial, Alkek building, room 740. These tubes will have been received within 24 hours of the blood draw and were shipped at ambient temperature. Pick up samples daily at 8:30 am.

2. After you return, verify that samples inside shipment were received in good condition, and that they match the Daily Biospecimen Collection form. Log and note any discrepancies into the Excel file at the link below via barcode scanning (includes time and date of receipt of sample).

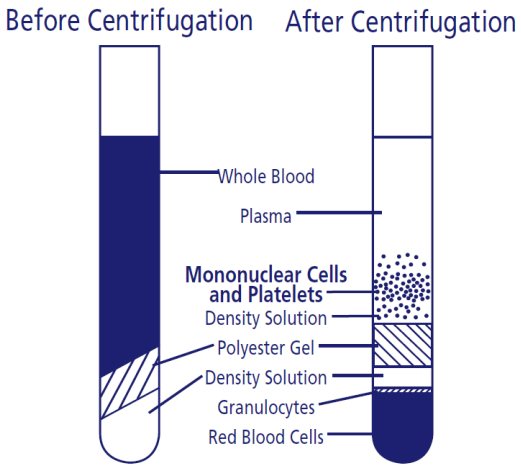
   \\Hgcnt2\ARIC_Visit5_Sample_Processing\Sample Receipt Logs\

3. If the coordinating center has not provided you labels, you will need to create labels using the Cryotags (cat. No LCRT-1200). Scan labels into the data source template found here: \\Hgcnt2\ARIC_Vist5_Sample_Processing\Labels\ARIC_Visit_5_Daily_Samples.xlsx. Then, open the ARIC Visit 5 Labels template in Bar Tender and print. The labels will print from the database automatically.

4. Remove Recovery Cell Culture Freezing Medium from the refrigerator and place in a dark area to warm to room temperature. (NOTE: Freezing Medium is light sensitive. Cover the medium container with aluminum foil and store this way at all times.)

5. In the tissue culture room, remove the contamination control mat layer every day and disinfect the hood completely including the face plate and the sides of the hood by spraying the inside with 3M HB Quat Cleaner and wipe the surface dry with kimwipe followed by 70% ethanol and wiping the surface dry with a kimwipe. Use the sterile lab coat only when working in the tissue culture room. Do not remove the lab coat from the room.

6. Samples received should have already been centrifuged. If not, then centrifuge for 20 minutes at 1650 RCF (Centrifuge program 6). If only using a small number of samples, put the tubes in the center of the baskets. After centrifugation is complete, remove the tubes and place them back in the hood.
   After centrifugation is complete, remove the tubes and place them back in the hood. All centrifugation steps should be carried out at room temperature (15–25°C) in a swing-out rotor. Note: the CPT should have a plasma layer, a white later of mononuclear cells and platelets, a gel layer, and red blood cells. You should not see RBC above the gel layer (see figure below). All samples transfer should be handled in the biological hood.



7. Mix by inverting the 4cc CPT tube 8 times.

8. Using a sterile transfer pipette, pipette all of the plasma and the white cell layer above the gel from the CPT tube into a 15 mL conical centrifuge tube for washing.

9. Using a serological pipette, add 1.0 mL PBS to the CPT tube. Tap the CPT tube 10 times and transfer the remaining liquid to the same conical centrifuge tube. Then, add PBS to the conical centrifuge tube to reach a total volume of 10 mL. Care should be taken not to touch the CPT tube or the conical tube. If you need to use your finger to balance the serological pipette when dispensing, do not touch it on the bottom half. This area enters the PBS bottle and could cause contamination. (Note: Discard any remaining PBS from the serological pipette do not add it back to the bottle.)

10. Cap the centrifuge tubes and mix by inverting 12 times.

11. First Cell Wash: Centrifuge the white cell conical centrifuge tubes for 15 minutes at 300 RCF (Centrifuge program 7).

12. Decant the clear supernatant from all the tubes (leaving about 0.5 mL) without disturbing the cell pellet. Re-suspend the cell pellet gently by tapping the bottom of the tube until sample is in solution. (Note: The cell pellet should be clear or light pink in color.)

13. Add enough PBS so that the total volume in the tube is 10 mL. Cap and mix by inverting the tubes 12 times.

14. Second Cell Wash: Centrifuge the white cell conical centrifuge tubes for 15 minutes at 300 RCF (Centrifuge program 7).

15. Decant the clear supernatant from all the tubes (leaving about 0.5 mL) without disturbing the cell pellet.
16. Using a sterile transfer pipette, add 1.0 mL Recovery Cell Culture Freezing Medium to each white cell centrifuge tube and mix by pipetting up and down 4-5 times. Then, transfer equal amounts of volume into each of the 2 PBMC aliquot tubes.

17. Return the Freezing Medium to the refrigerator for storage.

18. Place the PBMC aliquot tubes into a CoolCell controlled rate freezing container and store overnight at -80 ºC.

19. Return to the tissue culture room and remove all equipment from the hood. Disinfect all inside areas of the hood by spraying it with HB Quad disinfectant cleaner and wiping it dry with a kimwipe. Then, spray with 70% ethanol and wipe dry with a kimwipe. Check the reservoir in the hood, the floor, the centrifuge and all other work areas for blood products. If any blood products are visible, use the decontamination procedure stated previously.

20. On the following day (or on Monday for samples that were processed on Saturday), transfer the PBMC aliquot tubes to storage boxes in the -180 ºC Cryosystem (Liquid Nitrogen - LN) container for permanent storage. If using the electric LN freezer, verify that the Styrofoam tops or lid fits securely in the freezer. Remove any ice that has built up. Once the freezer is closed verify that the rubber gasket has a tight seal. Check that the liquid nitrogen tank is full. If the tank is almost empty contact Paula Johnson (713-500-9844) so that a replacement tank can be ordered.

21. Place all biological waste (blood associated items) in the orange biohazard bags in designated containers. When ready to dispose of all biological waste in the orange biohazard bags, the bags should closed with autoclave tape and processed each week using the autoclave in the SPH basement (Room B01A). Any person who is processing the waste should have taken the autoclave protocol class and have a current certificate before using the autoclave to sterilize biohazardous waste.

22. For any waste disposed of in the container with a red bag (non-blood associated items such as wrappers, used pipets), safety labels can be obtained from the UT Biological Safety cabinet in SPH_B01A, and should be completed according to instructions and placed on the boxes in the proper place. These waste containers are brought to and stored in this room until they are picked up by the contract vendor that UT Safety office has in place. ONLY the yellow labels should be used for our purposes as it ensures that the boxes are incinerated and not just thrown into a dumpster behind this building. If there are any problems with the boxes of waste being picked up or with disposal of autoclaved materials (housekeeping should be notified), call the Waste Management office at 713-500-5837 to leave a message for their further action on the situation.

8. MAYO CLINIC METHODS AND PROCEDURES FOR ARIC/NCS TESTS

9. SAFETY

MSDS documents are available for all chemicals (reagents) used at each respective laboratory facility.

APPENDICIES:

APPENDIX I: Table of Blood and Urine Specimen Collection. Aliquoting and Shipping Scheme

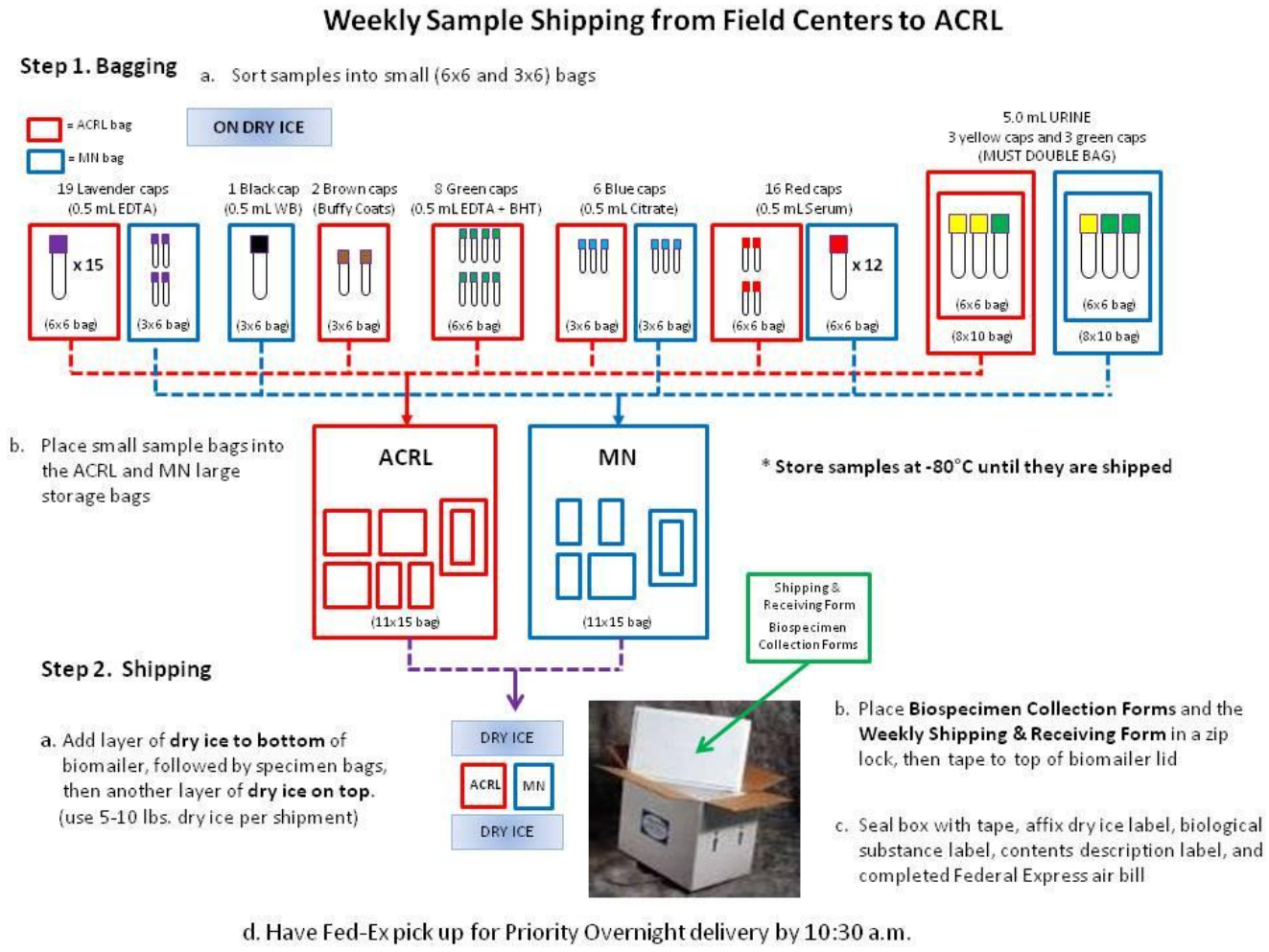
COLLECTION		PROCESSING			PREPARATION		
VENIPUNCTURE TIME	**IMMEDIATE ACTIONS**	**STAGE I**	**STAGE II**	**STAGE III**	**FREEZE**	**PACKAGING**	**Daily Shipping (Mon-Fri)**
Tubes 1,2 red/gray (SST) 10 mL RT	Mix ***5 x's*** and sit at Room Temp (RT)	Incubate UPRIGHT at RT 30-45 minutes	***Step 1, STAGE III:*** Centrifuge 10 min, 3000 g at room temperature ***Step 2:*** remove supernate from tube & place UPRIGHT in holding tube for aliquotting.	***Step 3:*** Using a repeater pipetor, transfer 0.5 ml serum from holding tube into (16) pre-labeled white vials with red screw caps	16 red top vials`	Place 4 red top vials into a 6x6 bag *labl'd w ID & to* ACRL (V-5/NCS) (12) 0.5 mL Red top vials into a 6x6 bag *labl'd w ID & to MN (V-5/NCS)*	NONE
Tube 3 (4 mL) CPT, Blue/Black -RT	Mix ***5 x's*** and sit UPRIGHT at Room Temp (RT)	UPRIGHT at Room (< 2 hours)	Centrifuge for 20 minutes @ 1800 x g (room temp). Invert 5x after centrifugation. Store at room temperature until shipping.			Place tube upright in shipping tube/rk for ARIC Genetic Lab	Ship daily to ACRL for pick up by ARIC Genetics Lab; directly to Genetics on Fridays. Use Refrigerant Packs at ambient temperature
Tubes 4, 5 (10 ml) 7 (4 mL) EDTA ***UNTREATED***	Mix ***8 x's*** and place in Ice Bath (Remove 0.5mL of whole bld. From tube 4-re-stopper). Place whole blood into white vial with black screw lid (tubes should be UPRIGHT)	***Step 1:*** Centrifuge 10 min, 3000 g at 4°C ***Step 2:*** remove supernate w pasteur pipet from ea tube & place UPRIGHT in holding tube (on ice) for aliquotting (KEEP TUBES 4,5 FOR STAGE II)	***Step 1*** : Transfer from tubes 4,5 white cell layers (buffy coat) into (2) pre-labeled white vials with brown screw caps.	Step 2: Using a repeater pipetor, transfer 0.5 mL EDTA plasma from the holding tube into (19) pre-labeled white vials with lavender screw caps. (Process on wet ice)	19 lavender top vials (EDTA plasma), 1 black top vial (A1c) and 2 brown top vials (buffy coats)	(15) lavender top into a 6x6 bag *labl'd w ID & to ACRL (V-5 & NCS)* & (2) brown top vials in a 3x6 bag *labl'd w ID to ACRL to ARIC Genetic Lab* , (3) lavender top vials into (1) 3x6 bag,*labl'd w ID & to MN & (1) lavender top vial into a 3x6 bag labl'd w ID & to Mayo (appropriate aliquots);* (1) black top vial into 3x6 bag *labl'd w ID & to MN (V-5 & NCS)*	**NONE**
Tube 6 (10 ml) EDTA ***TREATED w BHT***	Mix ***8 x's*** and place UPRIGHT in Ice Bath	***Step 1:*** Centrifuge 10 min, 3000 g at 4°C ***Step 2:*** remove supernate from tube & place UPRIGHT in holding tube (on ice) for aliquotting & adding BHT	No Activity	***Step 1:*** Using a repeater pipetor, transfer 0.5 ml plasma from the holding tube into (8) pre-labeled white vials with green screw caps. ***Step 2:*** Add 10µL of BHT (mix 3x) on ice	8 green top vials	(8) green top vials into a 6x6 bag *labl'd w ID & to ACRL (V-5)*	NONE
Tubes 8,9 (4.5 ml) Blue (Citrate) RT	Mix 8 ***x's*** @ Room Temperature (place UPRIGHT)	Incubate UPRIGHT at RT 30-45 minutes	***Step 1:*** Centrifuge w serum 10 min, room temp. ***Step 2:*** Remove supernate from each tube and place in a holding tube to aliquot (Rm. Temp)	Using a repeater pipetor, transfer 1.0 mL citrated plasma into (6) pre-labeled white vials, with blue screw caps	6 blue top vials	3 Blue top vials each into a 3x6 bag *labl'd w ID & to ACRL (V-5)* (3) blue top vials into a 3x6 bag *labl'd w ID & to MN(V-5 & NCS)*	**NONE**

COLLECTION		PROCESSING			PREPARATION		
VENIPUNCTURE TIME	**IMMEDIATE ACTIONS**	**STAGE I**	**STAGE II**	**STAGE III**	**FREEZE**	**PACKAGING**	**Daily Shipping (Mon-Fri)**
Tube 10, (2 mL) EDTA Hematology -RT for CBC	Mix 8x at RT (place tube UPRIGHT)	UPRIGHT at Room Temperature hold for daily shipping				Place tube in 3x6 bag or holder for ACRL (V-5)	Ship daily to ACRL for ACRL except Fri. ship to Genetics Lab for ACRL. Use Refrigerant Packs at ambient temperature
Tube 11, (2.5 mL) PAX (RT) Hold tube vertically below arm for draw & allow at least 10 seconds for the blood draw to take place. The blood will slow from a stream to a drip. Ensure that the blood has stopped flowing before removing the tube from the holder.	Mix 8x at RT (place tube UPRIGHT)	UPRIGHT at Room Temperature (must sit at least two hours), until shipped				Place tube upright in shipping tube holder. Put holder in biomailer for ARIC Genetic Lab. Use refrigerant pks @ ambient temp.	Ship daily to ACRL for pick up by ARIC Genetics Lab; directly to Genetics on Fridays. Use Refrigerant Packs at ambient temperature

URINE COLLECTION AND PROCESSING FOR ARIC VISIT 5/NCS

COLLECTION		PROCESSING			PREPARATION
Collect 30 mL Urine	Refrigerate	***Step 1:*** Pour into graduated cylinder or 50 mL centrifuge tube ***Step 2:*** Mix by inversion 2 x	**NON_ADJUSTED pH:** From graduated cylinder or 50 ml centrifuge tube, use a repeater pipet to transfer **5.0 mL into each of 3 vials with yellow tops**	**ADJUSTED pH**: ***Step 1:*** From the graduated cylinder or 50 mL centrifuge tube pour 15 mL into a beaker & adjust pH to 7(see urine ph adjustment procedure) ***Step 2: Using a repeater pipetor transfer 5.0 mL of the adj. urine into*** ea of 3 pre labl'd vials w green tops	Place 2 yellow tops and 1 green top into a 6x6 bag labl'd w ID & for ACRL; place 2 green top & 1 yellow top into 6x6 bag labl'd w ID & for MN. **Store in freezer til shipment**

APPENDIX II: Diagram of Packing Specimens At The ARIC Field Centers To Ship To ACRL



Appendix III. Example of a Completed Weekly Biospecimen Shipping Form

Weekly Biospecimen Shipping and Receiving Form

Batch ID Number:	F	0	0	0	1	2	4	**Form Code:**	W	S	F	Version: Revised: 05/24/2011

Instructions: Part 1 of this form is to be completed by the field center staff to document the **Weekly** shipping of the biospecimen collection to the ACRL. Scan the participant ID to auto-fill the ID number into your Data Entry System. Double-check participant ID # documented on this form with ID # of each sample during the packing of specimens (blood and urine samples inclusive).Part 2 is to be completed by the ACRL and UMN staff upon receipt of the shipment.

Part 1: Shipping (to be completed at the field center)

From:

Forsyth County		**Minneapolis Townships**	X
Jackson City		**Washington County**	

To: Charlie Rhodes
Baylor College of Medicine
CORE Atherosclerosis Laboratory
6565 Fannin Street, Room F-740
Houston, TX 77030

Staff Initials (shipping):	M	J	K	Date Shipped: (MM/DD/YYYY)	0	7	/	2	4	/	2	0	1	1

Number of Pages Attached: 4

Time Shipped: 0 8 : 4 5 (HH:MM in 24 hr. clock)

Field Center Comments: _____

Part 2: Receiving (to be completed at the ACRL lab)

ACRL Receiving

ACRL Staff Initials:	H	M	C

Date Received: (MM/DD/YYYY) 0 7 / 2 5 / 2 0 1 1

Comments: _____

Genetics Lab

Genetics Staff Initials:	F	R	S

Date Picked Up: (MM/DD/YYYY) 0 7 / 2 6 / 2 0 1 1

ACRL Shipment to UMN

Initials of Staff Shipping:	H	M	C

Date Shipped : (MM/DD/YYYY) 0 7 / 2 6 / 2 0 1 1

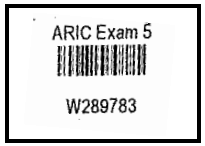
Scan the participant ID label for each collection of specimens and record the number of vials enclosed and condition code for each category (examples below) **before shipping and upon arrival**. (If more than one code for a specimen, choose "Other" and specify in a notelog).

Example of Complete Participant Sample	**Sample Condition Codes**

Tube #	**# of Vials**	**Cap Color**
#1, 2 (Serum)	16 (SR 1/16 – 16/16)	Red
#4, 5,7 (Untreated Plasma)	19 (UT 1/19– 19/19)	Lavender
#4 Red cells	1 (Hb A1c)	Black
#4, 5 (Buffy Coat)	2 (BC 1/2-2/2)	Brown
# 6 (Treated Plasma)	8 (T 1/8-8/8)	Green
#8, 9 (Plasma)	6 (P 1/6-6/6)	Blue
Urine	6 (Ur 1/6-6/6)	Yellow, Green

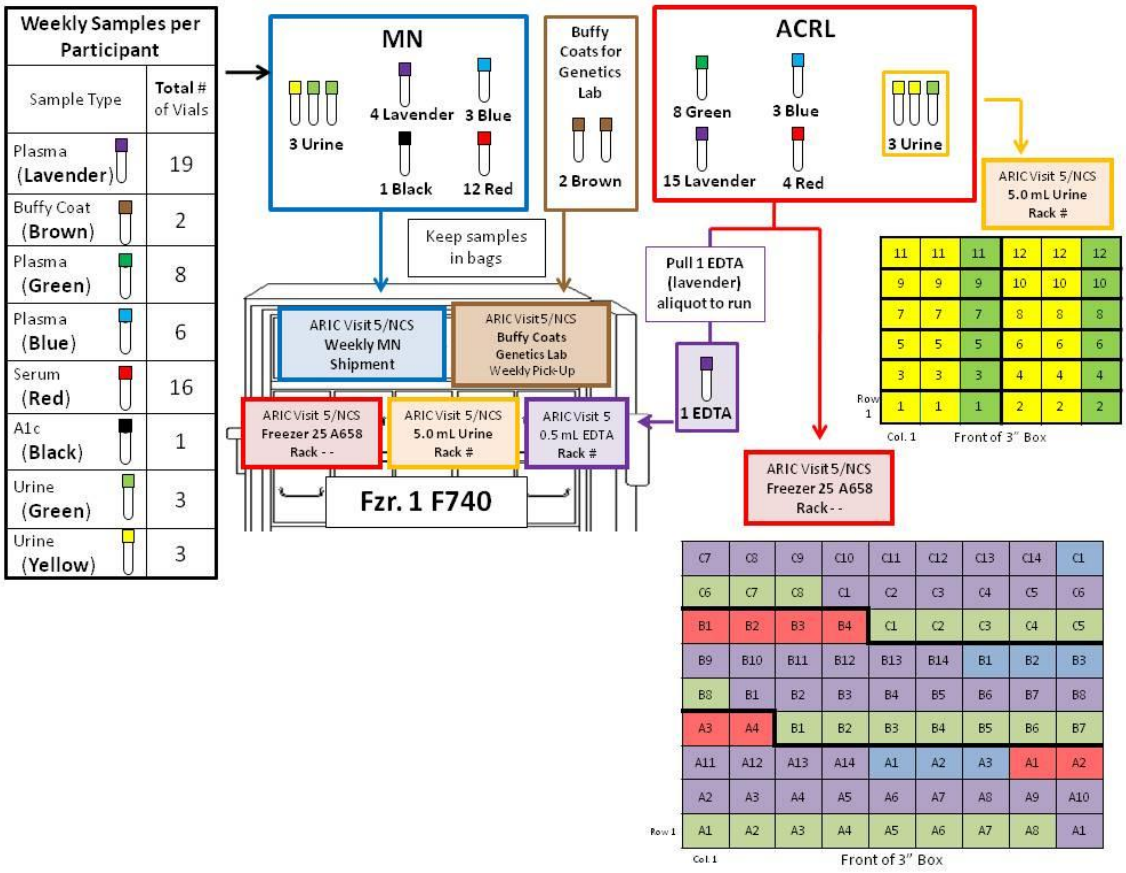
00 Good Condition	06 Hemolyzed
01 Thawed	07 Lipemic
02 Warm	08 Short Sample
03 Broken Bag/Vial	09 No Sample
04 Missing Label	10 Other on arrival
05 Other on shipping	

1. First Participant ID:



	Shipping			Receiving			
Type (Cap Color)	**# Vials Shipped**	**Condition Code** (Shipping)	**Field Center Comments**	**# Vials Received (ACRL)**	**Condition Code (ACRL)**	**# Vials Received (UMN)**	**Condition Code (UMN)**
Plasma (Lavender)	19	00		19	00		
Buffy (Brown)	2	00		2	00	Genetics Lab	
Plasma (Green)	8	00		8	00		
Plasma (Blue)	6	00		6	00		
Serum (Red/Gray)	16	00		16	00		
A1c (Black)	1	00		1	00		
Urine (Green)	3	00		3	00		
Urine (Yellow)	3	00		3	00		

Appendix IV. Weekly Processing



Appendix V. Specimen Condition Codes

Specimen Condition Codes			
00	Good Condition	06	Hemolyzed
01	Thawed	07	Lipemic
02	Warm	08	Short Sample
03	Broken Bag/Vial	09	No Sample
04	Missing Label	10	Clotted
05	Other (specify)		

Appendix VI: Weekly (Frozen) Aliquots Per Participant

Weekly Samples per Participant			
Sample Type	MN	ACRL	Total # of Vials
EDTA (Lavender)	4	15	**19**
Buffy Coat (Brown)	-	2	**2**
BHT-Treated (Green)	-	8	**8**
Citrated (Blue)	3	3	**6**
Serum (Red)	12	4	**16**
A1c (Black)	1	-	**1**
Urine (Green)	2	1	**3**
Urine (Yellow)	1	2	**3**