SECTION 9. CENTRAL BIOCHEMISTRY LABORATORY SAMPLE COLLECTION AND HANDLING

9.0.1 Calculation of Sodium, Potassium, and Protein Intake, Protein/Creatinine Ratio and Creatinine Clearance

Calculation of sodium, potassium, protein intake, protein/creatinine ratio and creatinine clearance is based on the 24-hour urinary excretion of sodium, potassium, urea, protein and creatinine respectively. The 24-hour urine collection Form #21 will report the 24-hour sodium, potassium, urea, protein and creatinine appearance in the urine.

The sodium excretion will be reported as grams per 24 hours and as millimoles per 24 hours.

The potassium excretion in the 24-hour urine collection will be reported as grams per 24 hours and millimole per 24 hours.

The protein excretion in the 24-hour urine collection will be reported as milligrams per 24 hours.

The creatinine excretion in the 24-hour urine collection will be reported as milligrams per 24 hours.

The 24-hour urine urea appearance will be reported on the 24-hour urine Form #21 as grams per 24 hours. The estimated protein intake can be calculated using this number in the following formula ([0.031] x [weight in kg] + 24 hour urinary urea appearance) x 6.25. Divide by the weight in kg = estimated protein intake.

9.1 Sample Processing at the CBL

9.1.1 Sample receipt and log-in

CBL Technician Responsibility

a. Record the receipt of the sample in the CBL sample log-in book.

b. Check Forms 22 and 23 and all sample tubes closely for any errors, discrepancies or other problems. Record these in the Problem Log (Section 9.7).

c. Notify the clinical centers by FAX, phone or electronic mail of any problems.

d. All analyses should be completed within three business days following receipt of the samples.

e. Any samples which are left over after analyses are completed will be kept refrigerated, space permitting, until the results are reported and reviewed by the clinical centers.

9.1.2Sample Storage at the CBL

a.Urines

1.Refrigerate urine samples until the time of analysis for creatinine, potassium, protein, sodium and urea nitrogen.

2.Label and freeze urine sample (7.2 mL) for storage at -70·. (B1, FV12 and annually)

b.Serum

1.Refrigerate serum for analysis of sodium, potassium, chloride, CO_2, glucose, BUN, calcium, magnesium, total protein, alkaline phosphatase, albumin, bilirubin, creatinine, HDL-cholesterol, LDH, phosphorus, AST, total cholesterol, triglycerides, GGT, uric acid and LDL as indicated until time of analysis.

2.Label and freeze serum sample (3.6 mL) for storage at -70·C. (G1, FV12 and annually)

c.Buffy Coat

1.Label and freeze buffy coat sample for storage at -70·C.

9.1.3Sample Distribution by the CBL Technician

a.Run QC appropriately or as procedure manual requires.

b.Label tubes with ID name code, number, date of the sample, and visit.

c.Put required amount of sample in each tube.

d.Urine samples for urea nitrogen must be diluted 1:11 with Saline diluent.

e.Hand carry the samples to the appropriate areas in the laboratory.

1.Serum:

a.Hitachi 747 and CX3 Delta CHEM-18 PANEL, Cholesterol, HDL-cholesterol, triglycerides,- 5.5 mL.

2.24-hour Urine Aliquot:

a.Beckman CX3 Delta - Creatinine, Potassium, Sodium-5 mL urine Aliquot; and Urea Nitrogen, 2 mL of urine dilution.

b.Manual section - Protein (TCA-Ponceau S method) - 5 mL of urine aliquot.

9.1.4 Analytical Methods of the Central Biochemistry Laboratory

a.Serum

1.Chem Panel and lipid profile: BMD/Hitachi 747 and CX3 Delta: See 747 Operations Manual and CX3 Delta Operations Manual. Note: creatinine measured by rate-Jaffe' methodology.

2.Total Cholesterol and Triglycerides: Hitachi 747 Bichromatic Analyzer using BMD Enzymatic Assay systems. See Naito, H.K. and David, J.A. Lipid Research Methodology 1-76(1984), Alan R. Liss, Inc., New York.

3.HDL-cholesterol: Hitachi 747 using the Dextran Sulfate -MgCl2 Precipitation Method. See Warnick et al., Clin. Chem. 28:1379(1982).

4.LDL-cholesterol: The following calculation is used for LDL. LDL = cholesterol - [(Triglyceride divided by 5) + HDL].

b.Urine

1.Protein: TCA-Ponceau S. See Pesce, MA and Strande, CS, Clinical Chemistry, 1973; 19-1265.

2.Sodium, Potassium, Creatinine, and Urea: Beckman CX3 Delta methods. See Beckman CX3 Delta Operations Manual. Note: creatinine measured with rate-Jaffe' methodology.

c.Result Report

1.The technicians who run the Hitachi and Beckman, analyzers will return the samples along with the results to the CBL technicians.

2.The CBL technicians run the urine protein assay.

3.Record the QC and precision results in the core lab QC book.

a.Review QC results.

b.If out of range, write up discrepancy report and take corrective action, in accordance with CCF QC guidelines.

4.Calculate proteins to mg/dl.

5.Write up Forms 18 and 19 for each patient, as needed. Make a highlighting line over area in log-in book when Forms 18 and 19 are completed.

6.All calculations and data records are visually rechecked for transcription errors.

7.Forms 18 and 19 is transmitted electronically to the DCC and Reports 20 and 21 to the clinical center. Record the data sent to the DCC in the log-in book.

8.Keep the original result print out.

9.2 External Quality Control for Clinical Center Laboratories

(intentionally missing)

9.3 External Quality Control Protocol for the CBL

9.3.1 Duplicate Samples

Every six months, each clinical center will send one duplicate sample to the CBL utilizing the QC ID names and numbers provided by the DCC. See Section 10.6 of the Manual of Operations for CBL quality control sample collection instructions.

a.The CBL will analyze the samples in the same manner as patient samples.

1.Analyze serum for Chem-Panel and Lipid Profile.

2.Analyze urine for creatinine, potassium, protein, sodium, and urea nitrogen.

b.Result Reporting

1.Report results in the same manner as for patients.

2.Complete Form 18 or 19, and handle as described in the section on patient result reporting.

c.Review of Results

1.The DCC will prepare a report comparing the duplicate QC results.

2.The report will be sent to the Clinical Center as well as the CBL Principal Investigator for review.

3.Results will also be reported in the QC Summary report for the Quality Control Committee to review.

a.Results which vary by a defined variance will be reviewed by the CBL technician.

b.On-going discrepancies and problems with reproducibility will be monitored by the CBL technician and Principal Investigator.

9.4Analysis of Discrepancies Found on Split Patient QC Samples Analyzed by the CBL

The purpose of this section is to define when CBL generated patient results should be deleted from the database.

Discrepancies discovered by analysis of duplicate QC samples must be investigated. The following are guidelines for determining which results are valid. The CBL Principal Investigator will review all discrepancies and make the final decision based on these guidelines.

9.4.1Patient and duplicate QC results

Patient and duplicate QC results which differ by an amount greater than the defined variance are to be rechecked.

a.If the recheck results are within the defined variance of the actual patient, the original results will be kept in the database.

b.If the recheck results are within the defined variance of each other but either one differs from the actual patient result by more than the defined variance, the original result on the actual patient will be deleted from the database.

c.If the recheck results differ by an amount greater than the defined variance, then the original result on the actual patient will be deleted from the database.

9.4.2 Not enough sample to recheck

If there is not enough sample to recheck the results, the patient result will be deleted from the database.

9.4.3 Recheck results which differ by the defined variance

Recheck results which differ by the defined variance should be investigated to determine the source

of the problem. Examples of problems which may occur:

a.An incorrect result was generated from the instrument.
b.The aliquots of the actual patient and duplicate sample were not properly mixed.
c.The aliquots were not from the same sample.
d.The aliquots were not stored properly.

9.4.4All Forms 18, 19, 20, 21, 22 and 23 with deletions will be flagged

9.5Internal Quality Control Protocol for the CBL

Currently, about 15% of the patient sample load constitute QC samples. Usually there are two to three known concentrations from several vendors, depending on the biochemical constituent being measured.

9.6QC Action Guidelines

9.6.1Daily Quality Control Action Procedures

1.Two Controls

If two controls are used and if one control result is within the 2 S.D. limits and one control is between 2-3 S.D. limits, the run is acceptable. Results can be reported.

2.Two Controls

If two controls are used and if one control result is within the S.D. limits but one control is outside the 3 S.D. limits, OR if both results fall outside the 2 S.D. limits, the run is unacceptable.

a.Do NOT report results from the run.
b.Take corrective action that may include but is not limited to the following:
1)Re-running control(s); the results are within expected limits.
2)Re-running patient samples from an acceptable run; the results are within expected proficiency limits.
3)Re-running selected patient samples from the unacceptable run on an alternate instrument; the results are within expected proficiency limits.
4)Re-running control(s) and selected patient samples from the unacceptable instrument in which the control results are within the expected limits.
5)Re-running the entire unacceptable run on the same or an alternate instrument in which the control results are within the expected limits.

NOTE:Re-running of control(s) can include
1)The same control(s)
2)Freshly reconstituted control(s)
3)Control(s) from another section/area
4)Repeating the pre-step procedure with control(s)

c.Notify the supervisor/staff if the problem cannot be resolved.
d.Do not release patient results until the problem has been corrected and QC is acceptable for the

run or an alternative procedure is employed. Verify and record ALL corrective action (examples of corrective action documentation are listed at the end of the Daily Quality Control Action Procedure).

3.More than Two Controls

If more than two controls are used and if two (2) or more of the control values fall outside the 2 S.D. range or one (1) or more fall outside the 3 S.D. range, the run is unacceptable.

a.Do NOT report results from the run.

b.Take appropriate corrective actions as described in Section 2b.

c.Notify the supervisor/staff if the problem cannot be resolved.

d.Do not release patient results until the problem has been corrected or an alternative procedure employed. Verify and record ALL corrective action (examples of corrective action documentation are listed at the end of the Daily Quality Control Action Procedure).

4.One Control

The use of one level of control run once is acceptable for only electrophoresis. If the control result is outside the 3 S.D. limit, the method QC is unacceptable.

a.Do NOT validate the run. Discontinue running patient specimens until the method demonstrates acceptable QC.

b.Take appropriate corrective actions as described above in section 2b.

c.Notify the supervisor/staff if the problem cannot be resolved.

d.Do not release patient results until the problem has been corrected or an alternative procedure employed. Verify and record ALL corrective action (examples of corrective action documentation are listed at the end of the Daily Control Procedure).

Examples of Acceptable Documentation (lettered for coding purposes):

a.Run unacceptable

b.Run repeated

c.Rechecked control(s)

d.No patients reported

e.Repeated patients on alternate instrument

f.Performed patient rechecks on same/alternate instrument

9.6.2Shifts and Trends

If QC results indicate a shift and/or trend:

1.Take action which includes but is not limited to the following:
a.Investigate method and/or control performance
b.Recalibrate
c.Perform calibration verification
d.Check reagent integrity
e.Process proficiency specimens
f.Evaluate alternate control material
g.Verify control limits
h.Evaluate instrument performance
i.Contact manufacturer
j.Evaluate inter-method performance

2.If investigation indicates potential incorrect patient results, do not release patient results until the problem has been corrected or an alternative procedure employed.

3.Documentation
Document all summary and detail of correction action on the QC log sheet.
Retain all supporting documentation.

9.7 AASK CBL Problem Record

Date	Date Msg Sent	Center	Problem	Response	Tech

9.8 Reference Ranges For The Central Biochemistry Laboratory

Serum	Sex	Range	Units
T. Protein		6.0-8.4	g/dL
Albumin		3.5-5.0	g/dL
Magnesium		1.6-2.4	mg/dL
Phosphorus	(Adult)	2.5-4.5	mg/dL
Calcium		8.5-10.5	mg/dL
Creatinine		0.7-1.4	mg/dL
Glucose		65-110	mg/dL
Urea Nitrogen	M	10-25	mg/dL
Urea Nitrogen	F	8-25	mg/dL
Uric Acid	F	2.0-7.0	mg/dL
Uric Acid	M	3.0-8.0	mg/dL
T. Bilirubin		0.0-1.5	mg/dL
AST		7-40	U/L
LDH		50-210	U/L
Alk. Phos.	(Adult)	20-120	U/L
GGT	M	0-50	U/L
GGT	F	0-35	U/L
Sodium		135-145	mmol/L
Potassium		3.5-5.0	mmol/L
Chloride		98-108	mmol/L
Carbon Dioxide		24-32	mmol/L

24-Hour Urine		**Range**	**Units**
Creatinine	M	1000-2000	mg/24 hrs
	F	800-1800	mg/24 hrs
Urea Nitrogen		12-20	g/24 hrs
Protein		<150	mg/24 hrs
Sodium		0.9-5.1	gm/24 hrs
		40-220	mmol/24 hrs
Potassium		1.2-3.9	gm/24 hrs
		30-99	mmol/24 hrs

Lipid Reference Ranges	Age	Sex	Risk Classification: Desirable	Borderline High	High
Total Cholesterol	<20	M,F	75-169	170-199	≥200
	≥20	M,F	100-199	200-239	≥240
LDL-Cholesterol	<20	M,F	50-99	100-129	≥130
	>20	M,F	60-129	130-159	≥160
HDL-Cholesterol	All	M	>45	36-45	≤35
	All	F	>55	36-55	≤35
Triglycerides	<15	M,F	25-120	121-299	≥300
	≥15	M,F	30-200	201-499	≥500