Framingham Heart Study

Manual of Procedures

MOP-version 1.0

September 6, 2018

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Laboratory: Post-Analytical

Tracking of Revisions to this FHS Protocol MOP

Revised Section	Revision Author	Date (s) of Revisions; source	Approved by, Date	Revisions	Previous Pages #s section changed	Distribution Date

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1.0 Aliquot Storage

Principle

To ensure proper cryovial storage of FHS samples

- Aliquotted samples are allowed to freeze at -80^{o}C in racks for one hour before storing in cryoboxes.
- Samples are divided into 2 main section: serum & plasma
    1. Serum: fasting serum, urine, citrate plasma and peak serum samples
    2. Plasma: fasting edta plasma, RBC, BC and peak plasma samples
- Samples can be grouped together based on either sample volumes, sample type or PI requesting the sample
- Labels are placed on top and side of cryobox. The label that is place on the inner box designate the starting point for that box. Each label contains Box ID, sample type, study group, exam number and box number
- Boxes are filled starting from the front left hand corner, moving left to right, front to back.
- A dates are added to the label when the box is started and ended
- A cryobox storage record (WS.9) is filled out for every storage group
- All cohorts are filed independently of the other except for Kiel samples
- Full boxes go into a -80 freezer holding compartment until it can be scanned into stored sample inventory.
- Once scanned, the boxes are placed in a designated freezer compartment
- Regular sample boxes hold 100 cryovials except for BC which holds 81.
- Any samples missing from the day is documented on the laboratory worksheet.

Current collections

The following boxes are maintained for each current study.

Edta Plasma

edta 1 - 3
edta 4 - 6
edta 7
edta 8-12
edta 13-14
edta 15 (Residual)
edta L1-L4 (Lewis)
edta K1-K4 M (Kiel) both GEN3 AND OMNIG2 are stored together
edta K1-K4 F (Kiel) both GEN3 AND OMNIG2 are stored together
RBC 1-3
BC 1-2 (Store in 9x9 box)
BC 3-4 (Store in 9x9 box)

Serum

serum 1-4
serum 5 (Long)
serum 6
serum 7-8
serum 9-10
serum 11
ser K1-K2 M (Kiel) both GEN3 AND OMNIG2 are stored together
ser K1-K2 F (Kiel) both GEN3 AND OMNIG2 are stored together

Citrate
- Cit 1-2

Urine
- urine 1-2
- urine 3-4

Peak edta
- Pk edta L1-L4 (Lewis)
- Pk edta 1-3
- Pk RBC
- Pk BC (store in 9x9 box)

Peak serum
- Pk ser 1-4

(Edition: 05.21.18)

2.0 Assay Parameters

**Hb: 4-40 g/dL
**A1C: 0.3-2.6

Assay (tests per kit)	On Board Stability	Calibration Frequency	Analytical Measurement Range	Extended Measurement Range
Blood				
Albumin (300)	12 weeks	4 weeks; lot change; as needed	0.2 - 6.0 g/dL	0.2 - 30.0 g/dL
ALT (500)	12 weeks	lot change; as needed	5 - 653 U/L	5 - 6530 U/L
AST (500)	12 weeks	lot change; as needed	5 - 684 U/L	5 - 6840 U/L
Cholesterol (400)	4 weeks	lot change; as needed	4 - 769 mg/dL	4 - 7690 mg/dL
Creatinine (700)	8 weeks	lot change; as needed	0.17 - 24.90 mg/dL	0.17 - 1731.7 mg/dL
CRP HS (300)	12 weeks	lot change; as needed	0.15 - 17.72 mg/L	0.15 - 265.95 mg/L
Glucose (800)	8 weeks	lot change; as needed	2 - 716 mg/dL	1 - 1432 mg/dL
HbA1c (150)	4 weeks	29 days; lot change; as needed	3.4 - 17.2 % **	NA
HDL-D (200)	12 weeks	lot change; as needed	5-121 mg/dL	5-242 mg/dL
Triglycerides (250)	8 weeks	lot change; as needed	9 - 750 mg/dL	9 - 3750 mg/dL

(Edition: 9.14.17)

3.0 Stability

CFAS INFORMATION			
Name	Assay	Store at 2-8C	Store at -20C
CFAS HbA1c	HbA1c	2 days	90 days
CFAS Lipid	HDL-D	5 days	30 days
CFAS Protein	CRP	4 weeks	---
CFAS	All chemistry not listed above	2 days	30 days

CONTROL INFORMATION		
Name	Store at 2-8C	Store at -20C
Diabetes (HbA1c)	7 days	---
BR 1 & 2	7 days	30 days
Lipid	14 days	---
PN/PP PLUS	5 days	30 days
ClinChem Multi 1 & 2	5 days	30 days

(Edition: 9.14.17)

4.0 Chemistry Repeat and Call Levels

Assay	Reference Range	Repeat	Acceptable Delta	Alert Levels	Urgent
Cholesterol	Desirable - <200 mg/dL High - >240 mg/dL	**<120** mg/dL or **>350** mg/dL	Refer to Common Delta	Alert: >350 mg/dL	None
Triglycerides	Desirable - <150 mg/dL High - >200 mg/dL	**<40** mg/dL or **>300** mg/dL	Refer to Common Delta	Alert: >500 mg/dL	>1000 mg/dL
HDL-Direct	Desirable - >40 mg/dL	**<20** mg/dL or **>90** mg/dL	Refer to Common Delta	Alert: <20 mg/dL	None
Fasting Glucose	50 - 99 mg/dL	**<70** mg/dL or **>125** mg/dL**	Refer to Common Delta	Fasting, non-diabetic >200 mg/dL Non-fast, non-diabetic >200 mg/dL Diabetic (all) >200 mg/dL	Same as Alert Same as Alert >300 mg/dL
Serum creatinine	Male - 0.7 - 1.2 mg/dL Female - 0.5 - 0.9 mg/dL	**<0.5** mg/dL or **>2.0** mg/dL**	0.1 mg/dL	>2.0 mg/dL [Except for participants on dialysis]	Same as Alert
HbA1c%	(4.0%-6.0%)	Hemoglobin **<6.0** or **>30** g/dL HbA1c **> current highest calibrator** HbA1c% **<4.6%** **≥6.5%** Non-diabetic **>8.0%** Diabetic	0.3 %	Alert: Non-diabetic ≥6.5%	None
Albumin	3.5 - 5.2 g/dL	**<3.0** g/dL or **>6.0** g/dL	0.1 g/dL	Alert: <3.0 g/dL	None
ALT	Male - ≤ 41 U/L Female - ≤ 33 U/L	**>50** U/L	Refer to Common Delta	Male - >90 U/L Female - >57 U/L	>180 U/L
AST	Male - ≤ 40 U/L Female - ≤ 32 U/L	**>50** U/L	Refer to Common Delta	Male - >90 U/L Female - >57 U/L	>180 U/L
CRP	< 1.0 mg/L - low risk 1.0 - 3.0 mg/L - average risk >3.0 mg/L - high risk	**<0.15** mg/L or **>40** mg/L	if <10; 0.3 mg/L if >10; 1.0 mg/L	None	None

****If Glucose >300mg/dL or Creatinine >2.0mg/dL: Repeat with Serum and EDTA plasma**

5.0 Handling Abnormal Results

PRINCIPLE/PURPOSE
Guidelines on how to respond to values outside expected parameters

1. DEFINITION

1.1. Repeat Values: Values that fall above or below expected parameters are repeated for verification.
1.2. Call Values: Repeat values that are communicated to participant's health care provider (HCP).

2. DETERMINATION

2.1. All results obtained at the FHS Lab are compared to values listed on the Chemistry Repeat and Call Level SOP
2.2. Based on the values outlined in the Chemistry Repeat and Call Level SOP, the decision to repeat specific tests and/or contact a participant's HCP is made

3. REPEAT TESTS

3.1. Any chemistry test not repeated within the run is recorded on the Repeat Log.
3.2. These tests are repeated on the next successive run.
3.3. Results are recorded on the Repeat Log and on the Laboratory Worksheet.

4. CALL VALUES

4.1. Should a value require notification of a participant's HCP the following steps should be taken:
- Repeat the test for verification.
- Have another technician check results.
- Prepare an Abnormal Test Result worksheet.

5. PREPARING THE ABNORMAL TEST RESULT WORKSHEET:

5.1. **DO NOT WRITE THE PARTICIPANT'S FHS ID NUMBER ON THE ABNORMAL TEST RESULT WORKSHEET UNTIL AFTER THE INFORMATION HAS BEEN FAXED.**
5.2. Participant's Name, date of birth and the exam date
5.3. Test result values
5.4. HCP's name, phone and fax number
5.5. HCP's address
5.6. Person contacted/spoken to at the HCP's office
5.7. Technician's initials
5.8. Date phoned
5.9. Date faxed

6. CALLING THE HCP

6.1. **Locate participant HCP information in PTS under the Physician Tab.**
- Identify yourself as a laboratory employee of the Framingham Heart Study
- Request the name of the person you are speaking to
- Identify the participant as a patient of Dr. XYZ
- Explain that while we perform tests for research purposes only, the following abnormal test results came to our attention. (Tell them the results).

- Ask for a fax number to fax the results.
- Ask the physician's office to call us back if they do not receive the faxed results
- Be sure to include a FHS Laboratory Fax Coversheet (see WS.15.faxcover)
- Write C/F (called and faxed) on the bottom of the FHS Laboratory Test Result Sheet.

6.2. Add participant's ID to the Abnormal Call Record and make a photocopy. Place original in the participant's chart and store the copy in Results Called Folder.

7. CALL CATEGORIES

ALERT VALUES:

1. FHS laboratory staff calls and faxes abnormal results to the participant's HCP.
2. A letter will be sent to the participant informing them that a lab result "outside the expected range," has been sent to their HCP. This letter will be sent along with the routine lab results.

URGENT VALUES:

1. FHS laboratory staff calls and faxes abnormal results to the participant's HCP, as above.
2. Laboratory staff brings the abnormal call sheet and participant's chart to a FHS MD.
3. FHS MD informs the participant of abnormal results either in person or by phone. The FHS MD completes the referral tracking form in RedCap indicating that the participant was spoken to. The lab keeps a copy of referral tracking form with the lab call sheet.
4. If the FHS MD believes the result is medically urgent a call will be placed to the participant's HCP.

8. PARTICIPANTS WITHOUT AN MD ON FILE:

ALERT VALUES:

1. Write up an Abnormal Test Result Worksheet. Include the participants Framingham ID on sheet
2. Write across the top of the sheet in red – "Alert Value – no MD."
3. Keep a copy of this sheet in the "Results Called" file folder in the lab.
4. The FHS MD will be asked to speak to the participant if he or she is still in the building.
5. The MD reviewing the chart will add a comment to the letter that is sent to the participant.

URGENT VALUES:

1. Follow steps 1-5 as above (for Alert Values).
2. In addition, the FHS MD will be asked to speak to the participant; either in the research center or by phone. A comment about this conversation to will be added to the referral tracking form in RedCap. The laboratory will keep this form in the results called folder along with abnormal call sheet.

(Edition: 10.02.17)

6.0 Releasing a Run

Purpose: To ensure the accuracy of the test results by setting guidelines for QC and assay values acceptability.

1. DETERMINATION OF ACCEPTABLE RUN

QC values for each run must follow the Acceptable Parameter Quality Control guidelines listed.

1.1 **Accept Run:**

The following conditions must exist before a chemistry run is accepted.

- All controls are within 2 standard deviations of the mean.
- One control is within 3 standard deviations of the mean and all other controls are within 2 standard deviations of the mean. This condition is used only sparingly. The run may be accepted if the out of range control appears to be a random event.

Reject Run:

The following conditions would cause a chemistry run to be rejected.

- One control is greater than 3 standard deviations from the mean.
- Two or more controls are greater than 2 standard deviations from the mean.
- The same control is greater than 2 standard deviations for two consecutive days.

2. DETERMINING REPEATS / CALLS

2.1 All results obtained at the FHS Lab are compared to values listed on the Chemistry Repeat SOP.

2.2 All chemistry results that are run in duplicate or rechecked must repeat within an acceptable delta difference based upon the following table:

Common Delta Table

1-30	**1**		**251-300**		**8**
31-60	**2**		**301-350**		**9**
61-90	**3**		**351-400**		**10**
91-130	**4**		**401-450**		**11**
131-175	**5**		**451-500**		**12**
176-200	**6**		**501-550**		**13**
201-250	**7**		**551-600**		**14**

2.3 Based on the values outlined in the Chemistry Repeat SOP, the decision to repeat specific tests and/or contact a participant's Health Care Provider is made.

3. TRANSCRIPTION

- Chemistry results are transcribed onto the lab worksheets by the technician performing the testing.
- Testing technician initials the top of each run they complete.

4. COBAS REVIEW

All runs are reviewed by a senior staff member and/or supervisor to assure accuracy. The person reviewing cannot be the technician performing the test.

It is the responsibility of the reviewer to ask:

1. Are all the controls within the acceptable ranges?
2. Are all values within the acceptable delta range?
3. Were results transcribed correctly?
4. Have all necessary repeats been identified and recorded on the Repeat Log?
5. Do any results need to be reported to a participant's physician?

After the run is checked, the reviewer:

1. Updates repeat log, QC log, laboratory worksheets
2. Writes the report date on laboratory worksheet
3. Notifies the responsible tester of any corrections
4. Initializes the analyzer printout after placing a check mark on the run
5. Files all paperwork

(Version: 03.05.18)

7.0 Estimated Glomerular Filtration Rate

1. DEFINITION

- Estimated Glomerular Filtration Rate (eGFR) is a calculated result which provides a clinically useful measure of kidney function.
- eGFR is calculated from serum creatinine using the Modification of Diet in Renal Disease (MDRD) Study equation.

2. IDMS-TRACEABLE MDRD STUDY EQUATION

- GFR (mL/min/1.73m^2) = 175 x (S creat)$^{-1.154}$ x (Age)$^{-0.203}$ x (0.742 if female) x (1.212 if African American)
- Serum creatinine is mg/dL, to two decimal places
- African American status is self reported

3. PROCEDURE

3.1. Weekly, laboratory data from the daily cobas run is uploaded and read into a SAS program.

3.2. The SAS printout is compared to the completed labsheets for the week and any discrepancies are corrected. eGFR values <60 are highlighted on the SAS printout.

↓

Obs	rack	position	s_id	a_date	report_date	ope_id	hba1c	alb2	alt	ast	chol_m	trig_m	gluc_m	creat_m	egfr	dhdl	crp
1	50040	1	1-xxxx	2011/05/16 11:08	2011/05/17	ja	8.9	4.2	16	18	150	207	109	1.85	26.9	38	7.21
2	50040	2	1-xxxx	2011/05/16 11:08	2011/05/17	ja	5.9	3.9	15	18	133	115	100	1.53	43.8	46	0.70
3	50040	3	1-xxxx	2011/05/16 11:08	2011/05/17	ja	5.7	4.4	27	31	206	85	94	0.72	80.3	60	0.58
4	50040	4	1-xxxx	2011/05/16 11:08	2011/05/17	ja	5.4	4.4	29	21	174	61	113	0.85	94.3	64	1.30
5	50040	5	1-xxxx	2011/05/16 11:08	2011/05/17	ja	5.4	4.3	33	25	135	140	70	0.77	101.1	53	1.86

3.3. Data from the SAS output is loaded into RedCap.

3.4. Lab reports are printed using Crystal Reports. Every value that is reported to participants or MDs is checked against the SAS printout. All highlighted eGFR values are confirmed on the National Kidney Disease Education Program website; www.niddk.nih.gov/health-information/communication-programs/nkdep/laboratory-evaluation/glomerular-filtration-rate-

calculators/mdrd-adults-conventional-units. Discrepancies should be brought to the attention of the lab manager and the data manager programmer.

3.5. eGFR values > 60 (mL/min/1.73 m^2), are reported as > 60.

eGFR values < 60 are reported as the value as calculated.

4. REFERENCE RANGE

Chronic kidney disease is defined as GFR < 60mL/min/1.73 m^2 for ≥3 months.
(National Kidney Foundation KDOQI Guidelines)

(Edition: 03.05.18)

8.0 Calibration Verification

DETERMINATION

CAP Calibration Verification/Linearity Surveys provide specimens and statistical evaluations of the results for verification of current calibration settings as well as for assessing the analytical measurement range of the laboratory method. The CVL survey satisfies the requirement for scheduled calibration verification and verification of the analytical measurement range as specified in the CAP Laboratory Accreditation Program and current CLIA Regulations Section 493.1255 for most analytes.

DEFINITIONS

Linearity is assessed by testing levels of an analyte, for which the relationship of concentrations is known. The user establishes the range over which the test method demonstrates a linear relationship between results and linear concentration.

Calibration is the set of operations that establish under specified conditions the relationship between reagent system/instrument response and the corresponding concentration/activity values of an analyte. Calibration procedures in this laboratory are specified by a method manufacturer.

Calibration Verification denotes the process of confirming that the current calibration settings remain valid for a method. This can be done by assaying the current method calibration materials as unknown specimens and determining that the correct target values are recovered or assaying matrix appropriate materials with target values that are specific for the method. If the method manufacturer provides a calibration validation or verification process it should be followed. The procedure must include at least three levels of concentration: low, mid and high.

PROCEDURE FOR CAP CALIBRATION/LINEARITY SURVEY

1. Run specimens and report results in the same manner you would patient samples.
2. Mix vials by gentle inversion prior to opening. Do not use a mechanical mixer.
3. All testing must be completed within one hour of reaching room temperature.
4. Perform 2 assays from each vial within the same run. Two data points must be received for each solution.
5. At least five consecutive data sets must be submitted in order for an analyte to be fully evaluated.
6. After running samples the results are submitted on line through an established laboratory web account.
7. Results are analyzed by CAP and a detailed report is then made available to the laboratory.
8. Report will give target values, means and acceptable bias.

PROCEDURE FOR BIO RAD HBA1C LINEARITY SET

1. Using a volumetric pipette, reconstitute each vial with 0.5ml deionized water. Replace stopper and allow to stand for 10 minutes. Swirl gently several times before testing.
2. Run specimens and report results in the same manner you would patient samples.
3. Perform 3 assays from each vial within the same run. Three data points must be received for each solution.
4. After running samples the results are submitted on line through an established laboratory web account.
5. Results are analyzed by Bio Rad and a detailed report is then made available to the laboratory.
6. Report will give target values, means and acceptable bias.

EXCEPTIONS
If a test has three or more levels or calibration material (low, mid and high value) then a calibration does not need to be performed. The test does need to be calibrated every six months.

FREQUENCY
Linearity and Calibration Verification testing should be performed every six months and documented as to the date of testing and the particular analyte that was tested. If values obtained are acceptable, calibration is verified. If not, a new calibration is required and the test repeated.

(Edition: 11.03.17)

9.0 Proficiency Testing Failure

PURPOSE

The Framingham Heart Study participates in two external proficiency testing programs; College of American Pathologist (CAP) – General Chemistry, Hemoglobin A1c, Cardiac Risk as well as the Centers for Disease Control and Prevention – Lipid Standardization Program. Each of these agencies sends data back to the participating laboratories. In the event of an unsatisfactory result, documented follow-up is required.

1. PROCEDURE

1.1. Fill out a Proficiency Failure Checklist, noting type of proficiency, assay which received the unsatisfactory result and the name of the shipment (CAP: C-A, or CDC Pool series #).

1.2. Describe the unsatisfactory result; FHS result and target value.

1.3. Review the test results of the proficiency sample or samples.

- Check for clerical errors
- Check QC performance for the testing period (CAP – day), (CDC – quarter).
- Retest sample(s)

1.4. Describe any other issues that should be considered in evaluating the result(s) in question

- Instrument performance during the period in question
- Calibration status (cal factor, age of standard curve during run(s), set point adjustments)
- Assayed values vs. target values of the same assay within that survey shipment
- Results from the alternate proficiency survey from the same period
- Results from previous surveys (history is especially important for the CDC pools)
- Lot history for reagents, calibrators and/or controls

1.5. Conclusions – detail any recommended actions or follow-up. If no conclusion can be drawn, document as “reviewed – no explanation; no action taken”.

1.6. Review with director and testing personnel.

(Edition: 10.26.17)

10.0 Reports

PURPOSE:

Inform participants and their doctors of laboratory results related to exam visit.

1. RUNNING A REPORT

1.1. Reports for participant's chart

- Log into RedCap. Lab folder. Go to crystal report lab_lh9.rpt
- Enter a begin date and an end date in the "mm/dd/yyyy" format. OK
- Check to see if the participant name matches the first record. Hit print.
- Compare these reports with the values on the laboratory sheets and SAS output
- If there are any differences, edits will be made by the lab manager

1.2. Reprint any necessary reports.

- Go to crystal report lab_id_lh9.rpt
- Type in idtype, id, and exam. Ok. Print

1.3. Mailed Reports

- Go to Infoview. Lab folder. Go to crystal report lab9.rpt
- Enter a begin date and end date in the "mm/dd/yyyy" format. OK
- Check participant name. Replace plain paper with FHS letterhead paper. Print.

2. LETTERS

2.1. Letters are generated to inform participant that an abnormal result was reported to their doctor.

2.2. Reasons for letter generation

- A value falls above or below the alert/urgent range
- A non-fasting status

2.3. Letters are only sent out if a call was made or if an abnormal cell population was reported.

2.4. The number of letters generated is based on the number of doctors stated by the participant

3. Both copies of the reports and any corresponding letters are sent upstairs for distribution.

(Edition: 10.26.17)

11.0 Corrected Reports

PURPOSE

Corrected reports are very rare at the Heart Study due to the lag time between the blood draw and the release of results and the number of checks before release. If a question arises about the accuracy of a released report the following protocol is used.

PROTOCOL

1. The problem result(s) should be brought to the attention of the laboratory manager.
2. A review of the run(s) involved is conducted, including calibration, quality control, results for other participants obtained on the same run(s) and data transcription. Participant data from past exams may also be reviewed.
3. If repeat testing is deemed necessary, serum and plasma samples are retrieved from the freezers and re-assayed.
4. When the re-assays are complete, the data are reviewed by the laboratory director and manager and a decision is made regarding the necessity of a corrected report.
5. If a corrected report is required;
    a. New laboratory result sheet with the corrected value(s) is printed
    b. A letter to the participant is written explaining the change in laboratory value
    c. A letter is prepared for the participant's physician
    d. The corrected report and the letters will be mailed to the participant and the physician.
    e. A copy of the corrected report is attached to the original result sheet and placed in the participant's chart.
    f. The data is edited in the permanent table; offlip_all, colab_all or gen3lab_all.
    g. Inform the participant coordinator about the corrected report. She may choose to inform the participant before the letters are mailed. Ask her to add a brief comment to the PTS Comment Line.
    h. Place copies of original reports, corrected reports, letters to the participant and MD, and a brief explanation of the event in the folder "Corrected Reports" in the lab managers office (file cabinet #3 drawer #1).

(Edition: 10.26.17)