Framingham Heart Study

Manual of Procedures

MOP-version 1.0

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Patrice Sutherland & Myoshi Holden

Laboratory: Pre-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 Quality Manual

1. Introduction

The Quality Manual addresses all activities that are essential in the operation of the Framingham Heart Study (FHS) Laboratory. Quality Assessment/Control policies and procedures define the necessary elements for the FHS laboratory to generate information that is precise and accurate. In addition, FHS Lab policies quantify the quality of laboratory work and regulate corrective action when necessary.

2. Maintenance of the Quality Manual

The Quality Manual is maintained and updated by the Laboratory Supervisor. Revisions to the Laboratory Quality Manual will be made as necessary. An annual review will be performed by the Laboratory Manager and/or Laboratory Supervisor. The following process is required for all changes to the laboratory QA Manual.

- Laboratory personnel notify the Laboratory Supervisor that a conflict or problem exists and identifies what change is needed to the QA Manual.
- The Laboratory Supervisor makes a judgment on what action is to be taken and modifies the written SOP.
- The Laboratory Supervisor presents the amended SOP to the Laboratory Manager for approval.
- The Laboratory Manager reviews the SOP and may request further investigation, recommends revisions and/or signs the amended SOP.
- Once the final revision of the SOP is completed and signed by the Laboratory Director, the QA Manual is updated and distributed.

3. Responsibility

It is the responsibility of each individual in the laboratory to support the Quality Process. Each individual is responsible for the technical quality of their work and must be alert to problems and sources of error that could compromise the quality of data. In order to assist employees in establishing and maintaining a high level of responsibility and skills, the FHS Lab provides thorough training in all aspects of the Laboratory process.

4. Overview and Organization

The Framingham Heart Study (FHS) is a long term community health study which began in 1948 to identify factors associated with the development of atherosclerotic and hypertensive cardiovascular disease. While reports of some Framingham Study findings are sent to the participant's physician, no specific treatment or medications are recommended by the Framingham Study doctors.

Personnel comprising the FHS Lab are responsible for sample collection, processing, testing, result reporting and data management activities. In addition, an extensive sample repository is maintained on site.

Facilities:

Seven rooms are designated laboratory facilities

- sample receiving/shipping station (Room 120)
- sample processing area (Room 120)
- dedicated analytical areas – Analyzers (Roche/Cobas 501) (Room 122)
- sample Ultra-low temperature storage (Freezer Rooms 125 and 127)
- administrative Areas / PC access / Data Management (FHS-LAB4, FHS-LAB6, FHS-LAB7 and FHS-LAB8 in Room 122) (LABSRV AND JANUS in Room 124)
- JANUS liquid handling system (Room 124)
- Additional lab space (Room 126)
- office for administrative work (Room 128)
- offsite ULT freezer storage at Fisher Bioservices – Franklin, MA

5. Confidentiality

As an employee of The Framingham Heart Study it is imperative to protect the confidentiality of participant information. As a means of ensuring confidentiality, everyone who works at the FHS or uses FHS records and data must complete an on-line course and maintain certification in Human Subject Protection every 3 years.

Human Subject Protection: http://www.citiprogram.org

In addition, all employees must read "Rules to Protect the Confidentiality of Framingham Heart Study Participant Information" which is found in the Framingham Heart Study Employee Handbook (page 7).

The Laboratory takes special care to protect the privacy of participants by removing all potentially distinguishing identifiers from specimen containers and paperwork that are sent to outside collaborating laboratories or investigators.

6. Personnel Qualifications and Responsibilities

Personnel of the FHS Laboratory are qualified by a combination of education, experience and training. The Laboratory Director and the Laboratory Manager set and monitor these standards. Detailed job descriptions, resumes and training records are kept on file and are available for review. The following sections summarize individual responsibilities of laboratory personnel.

6.1. Laboratory Director

The Laboratory Director acts as consult for all technical and scientific aspects of the laboratory.

Responsibilities:

- oversees scientific aspects of the laboratory
- ensures the laboratory maintains required licenses and certifications
- ensures that the test methodologies selected are capable of providing quality results
- provides consultation as to the appropriateness of testing and interpretation of test results

- provides on-site, telephone or electronic consultation for overall operation and administration of the laboratory as needed
- provides direction for all technical staff involved in the receipt, handling, storage, preparation and analysis of samples as well as the preparation, review, submittal and archiving of analytical data
- ensures that all QC issues or complaints are thoroughly investigated and resolved

Requirements:
- Physician (MD) with current medical license
- 5+ years experience clinical chemistry research or method development
- 5+ years experience in Laboratory Supervision or Management

6.2. Laboratory Manager (Technical Consultant)

The Laboratory Manager provides supervision, technical leadership and direction for the overall operation and administration of the laboratory.

Responsibilities:
- may perform any of the responsibilities of the Laboratory Director
- select test methodologies appropriate for use in the Laboratory
- verify test procedures performed and establish the laboratory's test performance characteristics including the precision and accuracy of each test
- establish and monitor quality control program appropriate for the tests performed
- resolve technical problems and ensure remedial actions are taken whenever test systems deviate from the laboratory's established specifications
- ensure that participant results are not reported until corrective action has been taken and the test system is functioning properly
- collaborates with other medical/academic institutions and private industries
- works with investigators to identify new laboratory assays for the study
- prepares budgets
- assigns and coordinates work of laboratory staff
- directs FHS laboratory quality control program
- manage extensive repository of irreplaceable blood samples of the Framingham Heart Study

Requirements:
- Bachelor's degree Medical technology, biology, or chemistry
- 5+ years clinical laboratory experience
- 5+ years experience in Laboratory Supervision or Management

6.3. Laboratory Supervisor (General Supervisor)

The Laboratory Supervisor, under the direction of the Laboratory Director and the Laboratory Manager, provides day-to-day supervision and direction for laboratory operations and personnel including phlebotomy, specimen processing, testing, quality control and data management.

Responsibilities:

- maintain all testing procedures performed at the Laboratory in a Procedure manual that is kept updated and reviewed annually by the Laboratory Manager
- maintain records of testing personnel including education, licensure or certifications, technical training, in-service training, competency testing and testing experience
- maintain records for at least two years of all participant logs, QC reports, proficiency tests, and retired procedures
- maintains copy of CLIA certificate
- maintains records of quality assurance activities, problems and resolutions
- ensure quality control is performed on all testing methods
- coordinate internal and/or external proficiency test performance
- follow up on all unacceptable performance with corrective action documentation
- maintain a safe working environment for all personnel
- ensure all equipment, machines or instruments are maintained and are safe to operate
- keep records of applicable service and maintenance agreements and temperature records as applicable
- monitors Data Entry/Management activities for the laboratory
- assists in the maintenance of the extensive repository of irreplaceable blood samples of the Framingham Heart Study
- performs/Reviews high complexity assays
- responsible for training laboratory staff
- provides significant guidance and support to testing personnel for all phases of work scope including phlebotomy, testing, instrument maintenance, data management, and issues involving participant health and safety
- maintains supply inventory
- responsible for laboratory purchasing

Requirements:

- Bachelor's degree Medical technology, biology, or chemistry
- 5+ years clinical or research laboratory experience

6.4. Testing Personnel

Testing personnel are responsible for specimen acquisition, specimen processing, test performance and result reporting according to laboratory guidelines and procedures.

Responsibilities:

- follow the laboratory's procedures for specimen handling, tests, results reporting and maintain a log of participant test results
- perform testing procedures according to written guidelines in the laboratory manual
- perform QA and QC procedures according to guidelines in the laboratory manual including completion of all documentation
- maintain records that demonstrate that proficiency testing samples are tested in the same manner as patient samples; proficiency records must be logged into the participant testing log with the day's run and tested by the same person following routine QA and QC procedures

- follow the established corrective action guidelines and procedures whenever test systems are not within the laboratory's acceptable limits of performance
- capable of identifying problems that may adversely affect test performance or reporting of test results and either correct the problems immediately or notify the Laboratory Supervisor, or Manager

Requirements:

- Bachelor's degree or Certificate in Medical technology, biology, or chemistry
- 1+ years experience clinical laboratory experience

6.6 Clerk

The Laboratory Clerk is responsible for updating and maintaining a computerized inventory of stored blood samples in the Heart Study Laboratory. The clerk also performs a variety of data entry tasks.

Responsibilities:

- scan in bar-coded sample identifiers (id number, date, sample type) of aliquots of participant samples
- organize contents of several freezers (-20C and -80C)
- maintain records of inventory status
- record and monitor daily freezer temperatures
- generate labels for participant sample aliquot vials

Requirements:

- Some college: minimum of two years of math/computer courses
- Strong computer skills and knowledge of PC, Microsoft Access, Excel Programs

7. Training

The Laboratory Manager and Supervisor are responsible for thoroughly training all laboratory personnel. Specific training duties may be assigned to senior personnel. The trainers closely supervise the trainees during a probationary period.

Training Agenda:

- Verbal instruction on the procedures and responsibilities of the job duties
- Reading assignments of applicable instructional materials, including the QA Manual and Standard Operating Procedures
- Trainee observation of testing performed by senior personnel (see Testing Personnel Training Checklist)
- Trainee performance of testing under supervision of senior personnel
- Final review and approval of trainees progress by Laboratory Supervisor or Manager
- Six month competency evaluation

7.1. Annual Competency Evaluation

Annually, the laboratory supervisor evaluates testing personnel. Competency is demonstrated with blind sample tests (phantoms), observation, reading of SOPs and an annual performance review. The laboratory supervisor will maintain a record of these phantom results for all results we report out (Annual Phantom Test Results). Competency will be measured using these blind samples in conjunction with a review of the past year's performance. Based on this evaluation the lab manager may recommend retraining activities. (See Personnel Competency worksheet)

7.2. Continuing Education

Quarterly training sessions are provided by the Laboratory Director, or other FHS investigators or staff on a laboratory related topic. Attendance is expected for all laboratory personnel. Each staff member must attend three of the quarterly training sessions in the year.

8. Sample Collection and Processing

Participants in the Framingham Heart Study are scheduled for an examination by FHS personnel. Sample collection and processing criteria are determined for each study group prior to the exam cycle.

8.1. Participant Scheduling

Exams are scheduled by the participant coordinators. Participants are asked to fast for 12 hours before their appointment, unless there are medical reasons that make fasting inadvisable.

8.2. Vacutainer Labels

Labels used for specimen collection vacutainers are generated and placed in the participant's exam folder, which is assembled before the exam date. Generation of these labels is linked to the Roster of the Framingham Heart Study; therefore the integrity of the ID number is assured.
Labels include both the name and the Framingham ID number.

8.3. Stored and Fresh Sample Labels

Labels are generated for specimen aliquotting and laboratory paperwork the day prior to exam.
Each study group has a label procedure for each exam cycle. Vials are labeled the morning before specimen collection begins.

8.4. Blood and Urine Collection

At the time of the blood draw the phlebotomist asks the participant his or her name, and confirms the name and ID number on the labels. Participants are also asked to provide a urine specimen. This specimen is collected in a cup that is labeled with their name, ID number and the number void for the day.

8.5. Sample Rejection

To insure the integrity of collected samples the FHS Lab has strict policies for accepting specimens.

8.6. Sample Aliquotting

Serum, plasma, buffy coat, red blood cells and urine are aliquotted according to Aliquotting Standard Operating Procedures. Aliquots of serum, plasma, buffy coat, red blood cells, and urine are stored at -80^0 C for future use.

9. Analytical Methods

Fresh aliquots of serum and plasma are tested daily.

9.1. Standard Operating Procedures

Analytical methods chosen are determined prior to the exam cycle for each study group. Each analytical method routinely used is documented in the form of an SOP which contains complete detailed instructions to standardize the expected performance of the analytical method. Contents of a laboratory SOP are given in Appendix A. Any deviations from published methodology are documented in the SOP.

9.2. Analytical Methodology Verification

Before any analytical method is routinely used to generate data, the method is validated. Criteria used to validate a method consist of the following:

- method selection by the Laboratory Director, Manager and/or Supervisor
- testing of method verifying reporting limits, dynamic range, precision, and accuracy criteria
- data acceptance criteria must be approved by the Laboratory Manager
- final documentation of the method in a written SOP

10. Equipment Maintenance and Calibration

Instruments may require daily, weekly, monthly, quarterly, or even semiannual maintenance and/or calibration. If a problem arises which cannot be corrected by the instrument operator, then the Laboratory Supervisor is notified. The Supervisor will coordinate the necessary diagnostic and corrective measures to be implemented. The incident will be documented in the instrument problem log book.

10.1. Temperature Monitoring

The following temperatures are recorded daily: room temperatures, refrigerators, freezers and refrigerated centrifuges. Settings and alarms for all freezers are also checked and recorded. The Framingham Study currently maintains twenty-two ultra low temperature freezers (-80 C) and one standard freezers (-20 C). All freezers are monitored by Stanley

Security and Rees Centron Monitoring System. In addition twenty-one ultra low temperature freezers are maintained off site at Fisher Bioservices in Franklin MA.

- Detailed freezer monitoring protocols, freezer failure and power failure protocols are found in the Framingham Heart Study Laboratory Freezer Protocol Manual.

10.2. Emergency Generator

Emergency back-up power is supplied by a Kohler 40KW generator. This generator unit includes a 150 gallon tank for diesel fuel. This would supply 48 hours of back-up power. The Kohler generator is exercised once a week. The fuel level of the generator is checked monthly. A service contract is in place, which includes semi-annual maintenance checks by Bigelow Electric.

10.3. Centrifuges, Analytic Balance, Pipettes

Annual maintenance is performed on all centrifuges (speed and accuracy) by Alert Scientific. Pipettes are serviced annually by TTE. All calibration and service records are retained in the laboratory.

10.4. Analyzer Maintenance and Calibration

As integral components in the Laboratory's functioning, analyzers are maintained and calibrated with strict attention to manufacturer's instructions.

10.4.1. Maintenance

Maintenance activities are specific for each analyzer. See the Operator's Manual for specific instructions.

Roche/Cobas 501 Chemistry Analyzer

Maintenance procedures for this instrument are fully described in the manufacturer's operator's manuals. There is a schedule of routine, daily, weekly, monthly and quarterly maintenance. All maintenance is recorded on Maintenance Log Sheets, including scheduled maintenance, as well as trouble shooting maintenance and service calls.

10.4.2. Calibration

Calibration activities are specific for each analyzer. See the Operator's Manual for specific instructions.

Roche/Cobas 501 Chemistry Analyzer

All assays are calibrated at intervals recommended by Roche. Assays are calibrated on initial start-up, if there are concerns about a specific test and if calibration verification fails. Calibration records are maintained for each

assay. Calibration date, calibration factor(s), reagent lot number and calibrator lot number are recorded.

11. Inventory

11.1. Reagents

All reagents, calibrators and control materials received in the laboratory are entered into the Reagent Log. The name of the reagent, lot number, expiration date, date received, date reagent is put into routine use are recorded on these logs. All materials are removed from use on or before their expiration date. When new control material lots are introduced, the new lot and the old lot are run simultaneously, providing an overlap period, which is used to assess the new lot and assign target ranges.

11.2. Sample Storage

1.1.1. Inventory

The Framingham Heart Study maintains a large inventory of stored participant samples. All samples are inventoried with their locations.

1.1.2. Monitoring

All freezers are monitored 24/7 by two independent systems: Stanley Convergent System and Rees Centron Monitoring System. An action plan with a protocol call list used to contact individuals during a power outage or freezer failure. The call list includes the phone and pager numbers of the Laboratory Manager and the Laboratory Supervisor.

1.1.3. Emergency Precautions

A 40 kW Kohler diesel generator provides back-up power. The Study also keeps several
freezer compartments in various -80 C freezers empty at all times for use during a freezer failure.

12. Quality Assurance Reports

QA Reports are generated by the Laboratory Manager with assistance from senior staff. These reports are used in evaluating the overall QA Program, identifying problems and trends, and planning for future needs and requirements.

Reports will usually include the following:
- All audit results including any necessary corrective action required
- Performance evaluation results and commentary
- Problems encountered and corrective action taken
- Any significant QA problems encountered
- Comments and recommendations

13. Long Term Drift Control

The FHS laboratory has been collecting data for drift since 1995. We have used a series of volunteer pools and participant pools collected for specific target values. Pool samples are aliquotted and frozen at -80C. There are 3 pool samples which are analyzed monthly; serum and two HDL pools

- Serum pool – all serum/plasma chemistry tests
- HDL pools – HDL

Pool data is recorded in the Long Range QC notebook.

The FHS laboratory also uses a set of "normal serum" samples, purchased from ProMedDx. This sample set includes 10 mL aliquots on 40 normal volunteers. This set is used for repeat measures on for non-routine tests.

14. Internal "Bench" Quality Control

14.1 Control Materials

Commercial control materials are run with every set of participant samples. Observed values are compared to target ranges. This range is the target mean plus or minus two standard deviations. Means and standard deviations are calculated for each new lot of control materials based on data from an overlap period, during which the old lot and the new lot are run simultaneously. Lot changes are made as infrequently as possible. Reagent lot changes is usually more frequent. Five repeats (from a previous run) and five CAP or CDC samples are tested for all new lots of reagents.

14.2 Testing

All chemistry assays are run with control materials at two or three different levels. Control materials are handled identically to participant samples. Controls are run at the beginning of every set. Control results are reviewed by the laboratory supervisor or a senior laboratory technician before the participant results are accepted.

14.3 Acceptance Parameters

14.3.1 Accept Run:

The following conditions must exist before a 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.

14.3.2 Reject Run:

The following conditions would cause a run to be rejected.

- One control greater than 3 standard deviations from the mean.
- Two or more controls greater than 2 standard deviations from the mean.
- Same control is greater than 2 standard deviations for two consecutive runs.

14.4 Record Keeping

Control values from every set are recorded on QC datasheets, which are maintained for all assays. Also recorded on the QC sheets are reagent lot changes, calibrations, instrument maintenance and any comments about the run. In addition, the FHS Lab uses a software program, Unity Realtime, to record and generate statistics and Levy-Jennings charts for QC data. QC statistics for all assays are summarized weekly and reviewed monthly by the laboratory manager and laboratory supervisor.

14.5 Replicate Testing

Some chemistry assays which are reported to participants and their HCP's are measured in duplicate. The average of the two values is reported. We have established limits for acceptable differences between replicate values. If a pair of duplicates does not fall within the acceptable range, the participant assay is repeated.

- Table 13.5 details the acceptable variation between replicates for lipid, glucose, ALT and AST measurements.

Table 13.5 Common Delta

Range (mg/dl)	Difference
1 -30	1
31-60	2
61 -90	3
91 -130	4
131 -175	5
176 - 200	6
201 - 250	7

Range (mg/dl)	Difference
251 -300	8
301 -350	9
351 -400	10
401 - 450	11
451 - 500	12
501 -550	13
551 - 600	14

- For serum creatinine and albumin, the replicates must be within 0.1 mg/dl.
- For HbA1c, the replicates must be within 0.3%
- For CRP, the replicates must be within 0.3 mg/L if <10 and 1.0 mg/L if >10.

14.6 Internal Review

All runs are reviewed by a senior staff member to assure accuracy.

It is the responsibility of this reviewer to check:
1. Are all controls within the acceptable ranges?
2. Are all values within the acceptable difference 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?

Once the reviewer has checked the run, identifies any necessary changes, the following steps are followed:

1. Update repeat log, qc log, laboratory worksheet
2. Notify the responsible tester of any corrections
3. Initial the analyzer printout
4. File all paperwork

15 External Quality Control

The Framingham Study participates in two external proficiency testing programs, the College of American Pathologists and the CDC-NHLBI Lipid Standardization Program. CAP and CDC testing samples are run with regular daily sets of each analyte.

15.1 College of American Pathologists (CAP)

CAP provides a level of evaluation based on peer group comparison. See Table 14.1

Table 14.1 - Peer group target values and evaluation limits

Analyte	Target value	Evaluation Limit
Albumin	peer group mean	± 10%
ALT	peer group mean	± 20%
AST	peer group mean	± 20%
Cholesterol	peer group mean	± 10%
Creatinine	peer group mean	± 0.3 mg/dl or 15% (whichever is greater)
CRP	peer group mean	± 30%
Glucose	peer group mean	± 6.0 mg/dl or 10% (whichever is greater)
HDL	peer group mean	± 30%
Triglycerides	peer group mean	± 25%
HbA1c	accuracy based	± 6%

CAP Surveys are shipped various times a year.

15.2 CDC Lipid Standardization Program

Lipid standardization pools are shipped yearly by the CDC. Each shipment consists of quarterly sets of twelve specimens. One sample per week is run alongside the daily participant samples. Submitted data from each laboratory is compared to the analyte means from the CDC reference methods, (RV – reference value).

A statistical summary is returned to the participating laboratories. These reports include run data for all samples in the shipment, including statistical data and CDC reference values and acceptable deviations. See Table 14.2.

Table 14.2 – Reference Values and Acceptable Deviations

Analyte	Target value	Precision (SD)
Cholesterol	RV ±3%	100-149 mg/dl – 4 mg/dl
		150 mg/dl – 3% RV
HDL Cholesterol	RV ±5%	<40 mg/dl ±1.7 mg/dl
		>40 mg/dl ±4% RV
Triglycerides	RV ±5%	5% RV

15.3 Unsatisfactory Proficiency Test Results

If the Lab is notified of a proficiency failure, immediate action is taken to correct the deficiency. A checklist that guides review of potential process errors is completed. See WS.049. If all steps of the checklist are completed and no clear cause for the unsatisfactory score is found, the checklist comments will include a statement of review with no explanation. Should this occur more than two times consecutively, outside consultation is sought to identify and remedy the problem.

15.4 Storage of Proficiency Samples

Unused proficiency specimens are aliquotted into cryovials and stored at -80°C. All samples are labeled with the date of freezing as well as sample identification, name of proficiency program and original date of receipt of sample.

16 Quality Control System for Repeated Measurement (Reproducibility) – Phantoms

16.1 Phantom Selection and Identification:

Blinded repeat measurements of laboratory assays are made for quality control purposes. A new phantom is collected for every 20 participants. A phantom participant is created using extra samples drawn from several Heart Study participants. Phantom Log Sheets are used to create a record, matching the phantom ID to the ID's of the source participants. The bar-coded label for the phantom ID is affixed to the top of the form. As samples are collected for the phantom, bar-coded ID labels identifying the source participants are added to the log sheet. When complete, a phantom sample set will be identical to the sample set of a standard participant for that study (including serum, plasma, red cells and urine samples).

16.2 Phantom ID:

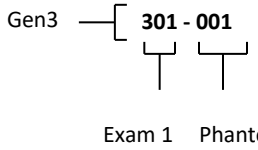
Phantom ID's are formatted in the following manner:

ABC - DEF

A = study prefix (Cohort = 0; Offspring = 1; Gen3 = 3; Omni = 7; Omni Gen2 = 72; NOS = 2)
BC = current exam number;
DEF = phantom number ordered consecutively within exam (1 – 999)

For example:

Phantom ID: 301-001 would indicate Gen 3 (3), Exam 1 (01) – Phantom #1 (001)



16.3 Record keeping

When each phantom is complete, with the full complement of tubes drawn and aliquots frozen, the log sheet is added to the Phantom Key file. The laboratory manager maintains a database, which links the Phantom ID to the source participant ID. Data are entered into the file using the bar-coded labels on the phantom log sheets.

16.4 Use

Phantoms are assayed along with participant samples as part of the daily laboratory runs. Phantom samples are also included in collections that are sent to collaborators and in sample sets that are frozen for future use.

16.5 Reporting

For in-house assays, the replicate data from the phantom and the true participant is linked by the Laboratory Manager. Phantom data is sent to the data manager for analysis.

17 Data Management

17.1 Controlled Documents

All documents in use must be approved by the laboratory director. Approved documents will bear the signed initials of the laboratory director. Signed originals are stored in binders that are maintained for accuracy and current version by the Laboratory Supervisor.

The master copies of Standard Operating Procedures (SOPs), Worksheets, Lists and Forms exist as computer files located at FHS-LAB4 in a folder called Current SOP (or Current Worksheets, Flowcharts). When a document is revised, the old file is moved into a file 'RETIRED' located on FHS-LAB4.

Documents which cannot be practically stored electronically, such as equipment manuals, are located on the bookcase in Room 122.

17.2 Records

17.2.1 Inventory

Samples are inventoried into the Inventory Database. This database is backed up daily by the IT department (on a main server).

17.2.2 Lab Reports

All data required to generate Lab Reports are loaded into a data management software called Clintrial by the Lab Manager. Lab Reports are generated weekly from data management software. These tables are backed up daily by the IT group. A SAS report is generated that is compared with the original runs. All corrections are highlighted, the correct value is written and is inserted into Clinitrial. At this time, lab reports can be generated. They are compared with each other as well as the original runs. If an alert/urgent action was taken, a letter notifying the participant will also be generated and included in the final report. Two copies of the lab report and any letters are sent upstairs for filing. A record stating the results were verified as well as HDL ratio and EGFR is checked and initialed.

Worksheets, raw data, calibration records and all other participant related records are stored in rooms 120-127 of the Laboratory.

18 Monitoring and Assessing System Problems

FHS recognizes that a system must be in place to monitor and assess potential problems that might occur within the analytical system. The following steps have been implemented to ensure minimization of potential problems:

18.1 Monitor

- Daily monitoring of each run occurs with the review of every run by the laboratory supervisor or a senior tech.
- Monthly formal review of QC occurs between the laboratory manager and laboratory supervisor. The review includes calculation of QC statistics, transmission of monthly QC to BioRad Quality Control Program (Irvine California), review of Levy-Jennings Charts, and review of peer group data.
- Proficiency testing results are reviewed as needed.
- Review of effectiveness of corrective actions taken which may include recalibration, range adjustment, review of peer group data and proficiency testing results
- Revision of polices or procedures to prevent recurrence of particular problems is also discussed during this review. If revisions are to be made the Laboratory Supervisor will update and/or revise the SOP Procedure Manual as needed.
- A review of all laboratory QC is performed by the laboratory manager in preparation for QC reports that are submitted to the NIH.

19 Corrective Action

FHS recognizes that problems occur in the normal course of work. Corrective action procedures are in place to control the effect of such problems on the data the laboratory generates. Records are maintained so that in cases of reoccurrence, the previous solution can be reviewed and considered.

Minor corrective actions are often in response to QC failures. These may include recalibration of an instrument, reanalysis of a batch of samples, application of instrument maintenance, or re-

preparation of a standard. Minor corrective actions are documented on the data sheet or in the QC log book for the analysis. The entry includes the following:

1. Description of the problem encountered.
2. Description of the action taken.
3. Evidence of resolution.

The Laboratory supervisor will decide if and when major corrective action is applied when a problem cannot be resolved immediately. The first step involves generation of a Corrective Action Report. Anyone in the laboratory can generate this report. Follow-up dates are established to evaluate the effectiveness of actions taken, and the findings are documented on the form. If it is determined that the problem has not been resolved, the process begins again to further investigate possible causes. Copies of the complete forms are kept on file for future reference.

20 Quality Control Review

Daily review

Each analytical run performed on FHS participant samples includes two or three quality control materials with target values at the low and high end of the expected range. Daily runs are reviewed by a senior level staff person (lab manager, lab supervisor, senior tech). Refer to the FHS Quality Manual, section 15, for a description of the quality control protocol and run acceptance parameters. Each run is initialed by the testing personnel and by the reviewer.

Weekly review

Weekly QC statistics are calculated by the laboratory supervisor, for all analytes. Assay means are recorded on the *Weekly QC Statistics Worksheet* (WS.40.QCweekly). Any quality concerns are brought to the attention of the laboratory manager.

Monthly review

QC data, and assay and instrument performance are discussed. Corrective actions are taken as needed. Notes are made regarding concerns or corrective actions taken. The *Weekly QC Statistics Worksheet* is initialed by the laboratory manager at the time of this review.

Using Unity Realtime (Bio-Rad Laboratories), monthly Levey-Jennings QC charts are created for all control materials. These charts are created on or close to the first of every month, for the previous month.

Quality control data is electronically transmitted to Bio-Rad, using Unity Realtime. This data transfer occurs on or close to the first of every month. QC statistics and peer data are returned in approximately two weeks.

The Levey-Jennings charts and Bio-Rad monthly QC statistics are reviewed by the lab supervisor and manager after receipt of the Bio-Rad data. Corrective actions are taken as needed. Notes are made regarding concerns or corrective actions taken. The Levey-Jennings charts are initialed by the lab manager at the time of this review.

Proficiency testing review

Proficiency testing data is reviewed by the laboratory supervisor and manager upon receipt of the PT reports. Corrective actions are taken as needed. Notes are made on the PT report sheets regarding concerns or corrective actions taken. The PT reports are initialed by the lab manager at the time of this review.

APPENDIX A

NCCLS GUIDELINE GP2-A3

Clinical Laboratory Technical Procedure Manuals

Each technical procedure should include explicit information and unequivocal instruction in the following areas wherever they apply. (The procedure manual should be easy to follow and contain all necessary information.) The NCCLS guideline contains several examples of procedure manuals for specific tests.

1. Test name, substance, or analyte tested.
2. Principle and/or purpose of test, which can be a short paragraph outlining the principle of analysis and the clinical reasons for performing the test.
3. Specimen requirements and collection and handling for the test. This includes any required patient preparation along with potential interferences from drugs and medications. Identify specific specimen requirements--type of collection container, amount needed, and criteria for unacceptable specimens. Timing considerations, transport/storage/preservation, and any special precautions must be identified. Procedures for submission of specimen to referral laboratory need to be included.
4. Reagents, standards, controls, and media used. Include source, directions for any preparation, and specific storage requirements. Label testing materials with name, lot number, concentration/titer, precautions, date prepared, and expiration date.
5. Instrumentation, including calibration protocols and schedules. Describe calibration protocols. This may mean following the manufacturer's instructions or following specific criteria established by the laboratory. If manufacturer's directions are NOT followed, state stepwise instructions including number, type, concentration of calibrators, acceptable limits, frequency, and reportable range for patient results.
6. Step-by-step directions including result reporting and troubleshooting and corrective actions.
7. Step-by-step instructions for any calculations. Show any equations in basic forms and show how to use each with specific examples. Identify how to handle variations such as dilutions.
8. Specific quality control materials to use. Give instructions for preparation and handling of the materials and identify the number and how frequently the controls are analyzed. For CLIA '88, the minimum requirement for most tests is two different concentrations every 24 hours. Analyte, manufacturer and/or laboratory quality control requirements can differ and each must be identified. State the tolerance limits for acceptable control values and corrective action to taken when the tolerance limits are exceeded. Describe how to record and store quality control data.

9. Expected values (normal range) and whether the range is age or sex dependent. List critical (panic) values that are life-threatening and a protocol for reporting these results.
10. Procedure notes, e.g., linearity or detection limit (reportable range).
11. Limitations of the method, e.g., interfering substances and/or pitfalls and precautions. Provide helpful hints, possible sources of error, alternate clinical applications, alternative procedures, etc. Include telephone numbers for technical service and reference pages from operator's manual for troubleshooting. Describe the action to be taken when the system is not operational.
12. Include references that can be from journal articles, textbooks, manufacturer product literature, technical/professional publications, research and validation data, and written personal communications.
13. Effective date.
14. Author.

REFERENCES

U.S. Department of Health and Human Services. Medicare, Medicaid and CLIA programs: Regulations implementing the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). Final rule. Fed Regist 1992; 57:7002-186.

(Version: 03.01.18)

2.0 Printing Labels

A. Weekly Schedule

1. Open Infoview => recruiting=>**bookingam.rpt** =>select date from calendar =>ok=>ok=>Print

Repeat until whole week's report is done

B. Weekly Sheet Labels

1. Open Bartender (FHS-DT-LAB SRV) => Gen3 OmniG2 exam 3 labels 0316
    - Enter framid with leading zeros into **Gen3 OmniG2 exam 3 participant log** table by date,

run **Gen3 OmniG2 exam3 participant query** to check names, and minimize screen

2. Open **Formats** under Bartender =>Open **sheet name,** print 80
   (Turn on printer HP Laser Jet P1102w and make sure sheet of label in printer face-up)

C. Weekly Platelet Labels

1. Open Bartender (FHS-DT-LAB SRV) => Gen3 OmniG2 exam 3 labels 0316
    - Under Tables
    - Open Platelet Plates
    - Enter dates for the following week

2. Open **Formats** under Bartender =>Open **platelet plate,** print 3

D. Daily Schedule

1. Open Infoview => recruiting=>**bookingam.rpt** =>enter date => ok on calendar => ok

    - Check the participant list, if there are any changes, print the list,
    - Log out (click **Log out** on upper right corner), and close the screen

E. Daily Labels Data Entry

1. Open Bartender (FHS-DT-LAB SRV)=> Gen3 OmniG2 exam 3 labels 0316

        - Under Tables
    - Open **date labels**, change date to tomorrow's date in mm/dd/yyyy (month and date without 0) format, change Kiel date to tomorrow's date in mm/dd/yyyy-50(month and date without 0) format, then close
    - Open **Gen3 OmniG2 exam 3 participant log**,
    - Enter record#:1, 2, 3…, framid, and date (mmddyy), leave menopausal and Kiel field empty

2. View Queries:

    - Open **Gen3 OmniG2 exam 3 participant query 0316**
    - Check framid, date, first name and last name against the daily schedule, then close
    - Open **Kiel Query M** and **Kiel Query F,** check framid, and names,

    click print preview, change margin to **wide**, print and close print preview

3. Under Reports:

    - Open **Mastersheet – Gen3 OmniG2 exam3**
    - Check framid, first name and last name, print and close
    - Close Access

F. Print Daily Labels

1. Large Labels printed using Lab7 computer

    - Open **Formats** under Bartender or LabelFormats-shortcut on desktop
    - Open **aliquot labels**, print 1
    - Open **Gen3 BC**, (**OmniG2 BC** or **NOS BC** if there are any), (record specid on the Spec ID sheet first)

print 5, and **save**, attach to **master label** by matching cell id

2. Large Labels printed using Lab6 computer

    - Open **Cobas creat**, print 1
    - Open **Cobas dups**, print 1
    - Open **Cobas fresh**, print 4
    - Open **fresh aliq,** print 1
    - Open **Immune-CPT** (record specid on the Spec ID worksheet first) print 2 and **save**
    - Open **iPS cells**, (record specid on the Spec ID worksheet first) print 2 and **save,**

      Attach to **master label** with **Immune-CPT,** by matching cell id
    - Open **master label**, print 2,
    - Open **Participant ID**, print 2, give to front desk receptionist
    - Open **Participant URL access code**, print 2, put with **Participant ID**
    - Open **test request** print 1

3. Small Labels printed using Lab6 computer

    - Change to smaller labels (press printer 2x to adjust label size),
    - Open **s exam date label**, print 4
    - Open **s Kiel date label**, print 1
    - Open **s name (small) label**, print 4
    - Open **s participant phntom,** print 1
    - Open **s platelet small**, print 1
    - Open **s DBS**, print 3

4. Box labels

    - Open **box labels** table under **Gen3 OmniG2 exam 3 labels 0316** folder, enter box #s,
    - Open **Formats**=>**box labels**, double click the field(s) you want to change, and then print 3

5. Phantom labels

    - Open Bartender (FHS-DT-LAB SRV)=> Gen3 OmniG2 exam 3 labels 0316
    - Open **phantom id** table

Enter new phantom id and date twice (leave the record# 1 and 0)

- Open **Formats** under Bartender

    Use Lab 7 computer) Open **phntom aliq**, print 1

    Use Lab 6 computer to print the following:

    - Open **phntom Cobas creat**, print 1
    - Open **phntom Cobas dups**, print 1
    - Open **phnton Cobas fresh**, print 2
    - Open **phntom fresh 110916**, print 1

Change to smaller labels

- Open **phntom Lab (small)**, print 1 (come out 2)

6. Cell line labels

    - Open Bartender (FHS-DT-LAB SRV)=> Gen3 omniG2 exam3 labels 0316
    - Open **cellline label log 0416** table, and enter framid and draw date (mmddyy)
    - Open **cellline query 0416**, check framid, date, first and last name, close it
    - Open **Formats** under Bartender
    - Open **c cell line name**, print 2
    - Open **c Gen3 cell line (c OmniG2 cell line, or c NOS cell line)**, record specid, and print 6 and save

(Edition: 12.07.16)

3.0 Sample Collection

PRINCIPAL/PURPOSE

Informational guide to Phlebotomy collection

1. Preparation

1.1. When exams are scheduled by the patient coordinators, the appointments are entered in INFOVIEW on the booking screen. Participant Research Center labels are generated prior to the day of the exam. These labels are created directly from information found in the official Roster of the Framingham Study Laboratory. Therefore, the integrity of the participant name and ID number is assured. Some of these labels are used on the blood collection tubes that are drawn in the Center.

1.2. On the day of the exam each participant is checked in and informed consent is obtained. The checkbox page along with a signature is placed in the chart. The entire consent is captured electronically on RedCap. Any negative responses will be documented by the laboratory staff on the phlebotomy log.

1.3. The laboratory staff will ask the participant to come into the examining room and have a seat on the bed. Introduce yourself and explain that you are going to be drawing their blood. Answer any questions that the participant may have about the blood draw. If you do not know the answer to a question, ask someone else. Common questions relate to the volume of blood taken, the tests we are planning on running and genetic research issues. Volumes of blood drawn are detailed in SOP. phlebotomy collection order which is posted in each drawing area. The volumes are given in ounces to give the participant a familiar reference point.

- Participants are asked to respond to the following questions.
- "Can you state your first and last name?" **Do not ask them, "Are you Mr. Smith?" Do not go by the name on their name tag.** Check the name against the name on the labels.
- "Are you fasting?" "What time was your last food or drink, not counting water?" "Have you had caffeine today?" Participants are asked to fast from 8:00 P.M. the evening before their appointment. Ask very specific questions about fasting status. Any caloric intake is considered a break in fast. Non-fasting status is recorded on the phlebotomy log and later noted on the laboratory worksheet. Tell center staff if a participant is non-fasting.
- "Are you a diabetic?" Inform the center staff if the participant is diabetic.
- See the PHLEBOTOMY SCRIPT for more information.

2. Phlebotomy

2.1. Venipuncture

- Participants are drawn in a supine position. Sleeves should be rolled up to expose the inner elbow.
- Put on disposable non latex gloves. Attach the Luer adapter of the butterfly needle to the Vacutainer holder. Wrap the tourniquet around the arm three to four inches above the venipuncture site. Identify the best available vein.

2.2. Cleanse the venipuncture site with an alcohol pad. Allow the area to dry to prevent possible hemolysis of the specimen and to avoid a stinging sensation to the participant when the needle enters the skin.

- Pull the skin taut to anchor the vein. Insert the needle with the bevel side facing up. Enter the vein using a smooth continuous motion.

- Place the appropriate Vacutainer (SOP.Phlebotomy collection order) into the Vacutainer holder. Grasping the holder securely, push the tube forward until the top of the tube is touching the end of the Vacutainer holder. Keep a slight forward pressure on the end of the tube (in the direction of the holder). If the Vacutainer tube does not fill completely, remove and try another tube. When the tube appears full, remove the tube from the Vacutainer holder.
- Once the first vacutainer begins to fill, remove the tourniquet from the participants arm.
- If the Vacutainer is NOT RED invert the tube gently ten times to mix blood with the anticoagulant. DO NOT INVERT THE RED VACUTAINERS!
- To remove the needle, lightly place a clean gauze pad over the venipuncture site. Remove the needle smoothly and quickly. Immediately apply pressure to the site with the gauze pad to prevent bruising. When the flow of blood has stopped apply a bandage.
- Dispose of needle still attached to the Vacutainer holder in the puncture resistant container.
- Dispose of all other contaminated waste in a biohazardous waste container.

2.3. Ask the participant to sit up on the bed. Have them sit for a few moments

- Label vacutainer tubes and ask participant to verify their name.
- When they appear to be ready to move onto the next station have them step down and escort them to the waiting room.
- Cleanse hands with hand sanitizer.
- Tubes are transported to the laboratory in an orange biohazard carrier.

2.4. Excessive Bleeding

- In the event of excessive bleeding or swelling, apply pressure to the puncture site for several minutes.
- If necessary, ask the participant to hold their arm in the air while pressure is applied.

2.5. Fainting

- Indications that a participant may faint:
  - Extreme paleness
  - Sweating
  - Dizziness
  - Nausea
  - Perspiration
- If a participant faints or you suspect they may:
  - Withdraw the needle.
  - Ask for assistance from center staff
  - Talk to the participant to divert their attention from the procedure and to keep them alert.
  - Keep the participant supine and have them breathe deeply.
  - Apply a cold compress or washcloth to the forehead and back of the neck.
  - When the participant begins to feel better offer crackers and or something to drink.
- Once the participant recovers they should remain in the area under supervision for at least 15 minutes or until they have fully recovered.
- Document the incident.

3. Urine Testing

3.1. Participants are asked to leave a random urine sample when they arrive in center. The samples are left in the restrooms until retrieved by laboratory personnel for aliquotting.

4.0 Phlebotomy Training

PURPOSE: To demonstrate phlebotomy skills

1. Readings
    a. SOPs
        - Sample collection
        - Phlebotomy Script
        - Phlebotomy Training
        - Phlebotomy Protocol
    b. Text in "Phlebotomy Essentials" textbook
2. Guidelines to FHS blood draw
    a. Consent process
        - Review informed consent
        - Read the consent form
    b. Test Request
    c. Phlebotomy log
    d. FHS blood draw
        - Fasting
        - Peak Exercise
        - Cell Line
    e. Participant Comfort
    f. Confidential comments
    g. Stocking consumables
    h. Safety, PPE, & Medical Waste
3. Direct observation
    a. Observe 15 fasting blood draws
    b. Observe 15 peak exercise blood draws
4. Perform
    a. Perform 5 non-participant volunteer blood draw
    b. Perform 20 fasting blood draws with supervisor observation
    c. Perform 20 peak exercise blood draws with supervisor observation
    d. Perform 10 fasting blood draws with supervisory feedback
    e. Perform 10 peak exercise blood draws with supervisory feedback
5. Test

(Edition: 11.7.17)

5.0 Phlebotomy Collection Order

PRINICIPAL/PURPOSE

Draw order and total volume for current exam cycle

Gen3 Exam 3 OMNI2 Exam 3 NOS Exam 3
1. EDTA 10 mL (lavender)
2. EDTA 10 mL (lavender)
3. Serum 10 mL (red)
4. Citrate 4.5 mL (blue)
5. Hirudin 3 mL (white)
6. CPT 4 mL (blue/black)
7. Citrate 4.5 mL (blue)
8. Citrate 4.5 mL (blue)
9. Serum 10 mL (red)
10. EDTA 10 mL (lavender)
11. EDTA 10 mL (lavender)
12. Citrate 4.5 mL (blue)
13. CPT 8 mL (red/green)
***If a cell line is required, draw 2 (8ml) CPTs before tube #2. Omit Tube 13.
Peak Exercise
1. EDTA 10 mL (lavender)
2. Serum 10 mL (red)

Draw all tubes in the order written.
EDTA tubes are labelled 1-4 based on the drawn order.
Serums tubes will have a 1S or 2S written on the label.
Citrate tubes for platelet aggregation are labelled 1-3 based on the drawn order.
Serum and Hirudin tubes have time of draw on label.

FASTING
Total volume = 93.0 mL (3.1 oz or 6.3 T)
Total volume = 101.0 mL (3.4 oz or 6.8 T) (with cell line)

PEAK EXERCISE
Total volume = 20.0 mL (0.7 oz or 1.4 T)

(Edition: 03.10.17)

6.0 Sample Processing

PURPOSE:
Demonstrate the components in the preparation of samples for analysis and storage.

1. LABELS

1.1. Microsample tubes are labeled with the barcoded labels the morning before exams begin. Apply the labels very carefully, aligning the label along the top of the tube and pressing down the edge where the label overlaps. SOP.PRINTING LABELS

2. SET-UP

2.1. Samples for long-term storage are stored in screw cap microsample tubes with an O-ring cap. There are various sizes of microsample tubes (SOP.ALIQUOTTING). Samples that will be assayed fresh are transferred into 5 ml polystyrene test tubes with push in stoppers.

2.2. Aliquotting rack set up

- Set up aliquotting racks before first participant of the day.
- Organize microsample tubes in racks according to the aliquotting diagrams found in the SOP.ALIQUOTTING. Each cohort group will have a different color rack.

3. HbA1c

3.1. Prior to centrifugation, allow EDTA tube #1 to rock for 5 minutes before transferring approximately 1 ml of whole blood into a 5ml barcode labelled tube. (SOP.PHLEBOTOMY PROTOCOL) Allow the 5ml to mix on the rocker until ready to run on the Cobas c501.

4. CENTRIFUGATION

4.1. Turn on the power to refrigerated centrifuges 30 minutes prior to use.
4.2. Red top serum tubes must sit at room temperature for a minimum of 30 minutes to allow for complete clotting. The time drawn is written on the label after phlebotomy.
4.3. Citrate and EDTA tubes can be spun immediately.
4.4. To balance the centrifuge, arrange the Vacutainers in the centrifuge carriers so that the carriers opposite each other are mirror images. Use Vacutainers filled with water if necessary. Lock the centrifuge safety shield into place and then close the cover.
4.5. There are 2 models of refrigerated centrifuges; Sorvall Legend and Eppendorf 5810R.

All tubes centrifuged for 22 minutes at 4C.
Sorvall: Program number 1 (3500 rpm / 2500 g)

Eppendorf: Program number 5 (3530 rpm / 2500g)

5. **URINES**

5.1. Aliquot approximately 1ml of urine into a 5ml labeled tube for pregnancy testing.

6. SAMPLE STORAGE

6.1. Samples saved for in-house testing are stored temporarily in ref5. Samples are saved until all laboratory results have been reviewed and released. Discard samples only after confirming that testing for each sample is complete.

6.2. Long term storage

- The Framingham Heart Study maintains a large repository of stored samples. We also have an offsite repository.
- Specimens currently collected at the Heart Study are stored at -80 C. Frozen specimens are stored in cardboard cryo boxes. These boxes hold 100 samples; (10 rows of 10). Boxes are filled starting from the front left hand corner, moving left to right, front to back.
- Each on-site freezer is assigned and labelled with a unique number (FREEZER PROTOCOL). There is also information about ID number, Stanley Security zone, Rees input, model number, serial number and date of purchase.
- The offsite freezers are located at:

Fisher BioServices
10 Forge Park
Franklin, MA. 02038
Contact persons: David Josephs (Operations Manager) 508-553-0414
Barbara Poulin barbarapoulin@thermofisher.com

6.3. Cycle collections

- There is at least one sample type from every cycle stored onsite, the rest are stored offsite to prevent loss of a sample set.

6.4. Records

- The Framingham Heart Study maintains complete records for all sample storage online.
- A folder for Cryo box records is kept for each exam.

(Edition: 03.03.17)

7.0 Sample Rejection

PRINCIPLE/PURPOSE

To define criteria that would require a collected specimen to be rejected

1. BLOOD

A sample will be rejected if the sample meets ANY of the following criteria:

1.1. Unlabeled

1.2. Hemolyzed

1.3. Stored inappropriately (i.e. frozen)

1.4. Inappropriate blood volume

- Note: Vacutainers used for the collection of venous blood contain additives in varying concentrations dependent upon the amount of vacuum and the required additive to blood ratio for the tube. Because of calculated additive ratios it is imperative that proper blood fill volumes are obtained.

Evacuated Blood Collection Tube	Expected Fill Volume (mL)	Acceptable Volume (mL)	Comments
EDTA (Purple top)	10	5	Discard if under filled
Citrate (Blue top)	4.5	4.5	Discard if under filled
CPT (Blue tiger top)	4	----	
CPT (Blue tiger top)	8	----	
CPT (Green tiger top)	8	-----	

►**Red serum vacutainers contain no additives and can be used regardless of fill volume.**

2. URINE

A sample will be rejected if the sample meets ANY of the following criteria:

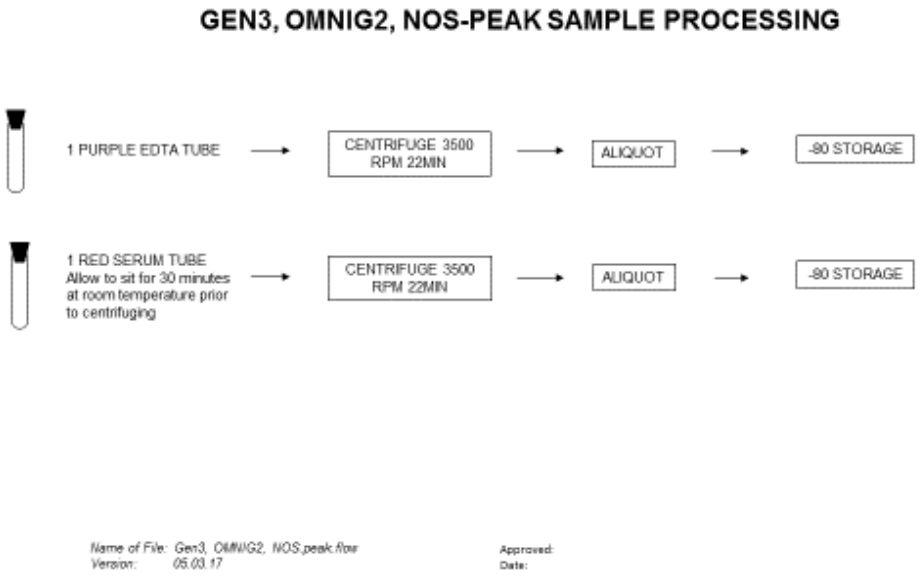
2.1. Unlabeled

2.2. Collected or stored inappropriately (i.e. frozen)

Note: If a participant has been taking medication that may affect the color of his/her urine (i.e. antibiotics), that sample will be tested, saved and noted on the Laboratory Worksheet. Any results obtained may be influenced by color changes.

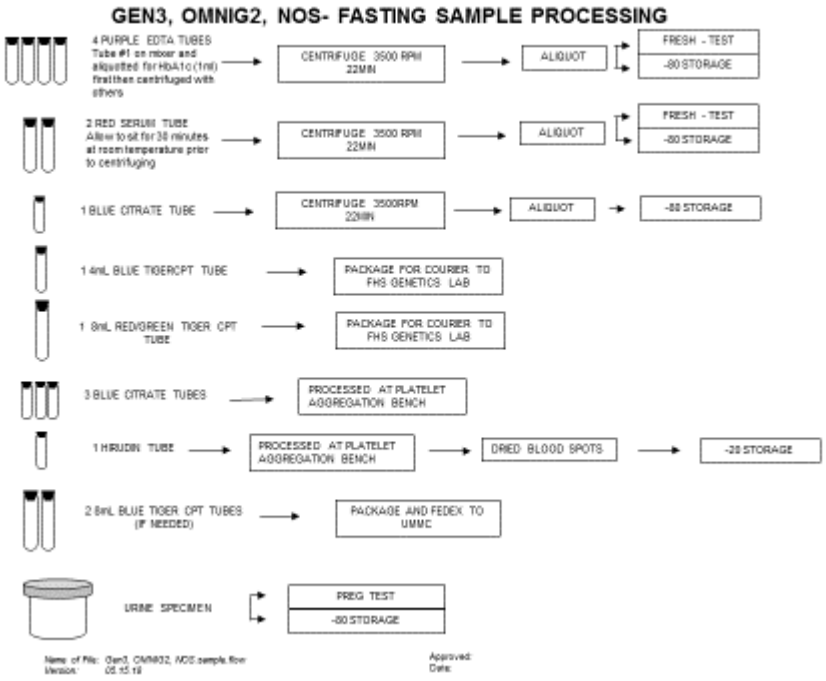
(Edition: 03.21.17)

8.0 GEN3, OMNIG2, NOS--Peak Sample Processing



(Version: 05.03.17)

9.0 GEN3, OMNIG2, NOS--Fasting Sample Processing



(Version: 05.03.17)

10.0 Aliquotting Procedure

EDTA 1 HbA1C FRESH EDTA 1-3 0.3ml EDTA 4-6 0.5ml BC 1 RBC 1	SERUM 1 FRESH SERUM 1-5 0.3ml SERUM 6 1.0ml or SERUM K1-K2 0.5ml SERUM 7 0.5ml
EDTA 2 EDTA 7 1.2ml or EDTA K1-K4 0.3ml EDTA L1-L4 0.3ml EDTA 8 1.5ml BC 2 RBC 2	SERUM 2 SERUM 8 0.5ml SERUM 9-10 1.5ml SERUM 11 Residual
EDTA 3 EDTA 9-10 1.5ml EDTA 13 1.0ml BC 3 RBC 3	
EDTA 4 EDTA 11-12 1.5ml EDTA 14 1.0ml EDTA 15 BC 4	
*EDTA 15 Residual-any plasma left over from tubes 2-4.	

11.0 Cell Line Shipment Preparation

Samples collected for cell line generation are sent via FedEx to Greg Rynders of Advanced Research and Diagnostic Laboratory.

Greg Rynders
University of MN-ARDL
1200 Washington Avenue South
Suite 175
Minneapolis, MN 55415
Phone: 612.625.5040
Email: rynd0021@umn.edu

4. Preparation

4.1. Blood collected in blue tiger CPT Vacutainers are separated from other FHS samples.
4.2. Print cell line labels and date labels (**SEE SOP.LABELS**)
4.3. FHS ID labels are removed from each Vacutainer and replaced with bar coded cell line labels. Usually two CPT tubes are drawn (where CPT's are requested).
4.4. A cell line name label is affixed to the master cell line log worksheet (**WS.4**) along with date label.
4.5. One cell line label is affixed to the master cell line log sheet and another to UMN-ARDL cell Line worksheet along with date label (**WS.4b**)
4.6. The total volume of blood, per participant, is recorded on both sheets.
4.7. The master cell line log sheet is used for data entry into the LIMS file in the laboratory computer. A second person initials the sheet after ensuring that the data was entered correctly. The master sheet is then filed away.
4.8. UMN-ARDL cell line worksheet is photocopied twice. The original plus one copy is sent out and the remaining copy is saved in the FHS Lab.

5. Packaging

5.1. Materials

- Primary receptacle = Glass Vacutainer
- Molded 8-tube foam mailer
- Absorbent material
- Press-lock poly bags
- Cardboard carton
- U-Tek® Gel packs
- Insulated bio-mailer

5.2. Paperwork: Paperwork is placed in a sealed press-lock bag for transport.

- Original UMN-ARDL Cell Line Log Sheet plus copy
- Diagnostic Specimens Declaration / Emergency Contact information.

5.3. Packaging Instructions

- Place re-labeled Vacutainers into absorbent foam lined cardboard box.
- Once filled, or when no more samples need to be packed, place each box into a press-lock poly bag and close.
- Place room temperature U-Tek® Gel packs into the bottom of an insulated bio-mailer.
- Place packed cardboard cartons into the bio-mailer. The bio-mailers fit two cardboard cartons.
- Insert paperwork into another poly bag and place bag inside bio-mailer.

- Place U-Tek® Gel packs on top of cardboard box.
- Return insulated lid to bio-mailer.
- Seal box with packing tape.
- Place Human Exempt Specimen label on two sides of the box.
- Generate FedEx label and affix to top of box. **SEE SOP FEDEX**.
- Place contact label on side of box.
- Take box to FHS reception area for FedEx pickup before 3:00 pm.

6. Data Entry

6.1. Participant data from CPT samples is scanned from the bar code labels on the Master Cell Line Log Sheet into an Excel workbook on FHS-Lab7 computer. The file is located at Lab7/LIMS/1 LIMS G3 ex3 0416/LIMS 031716.

6.2. For detailed instructions on entering this data see *Data Entry of CPT see the read me, when open the above file.*

6.3. Once file is saved, email the following people

- Greg Rynders
- Eileen Studt
- Vicky Makky
- Sue Blease
- Heather Arruda
- Jessica Rumpf
- Include in the email give information regarding the cohort and draw status (ie. 1st time draw), an attachment of cell line ship file, the fedex tracking number and our contact information.

6.4. Update PTS Tracking. Enter Participant ID. Go to Cell Tab. Enter the following information for first time draw:

C_TYPE:	0	
SAMPLE_DATE:	10/11/2006	MM/DD/YYYY
SAMP_OB:	5 (5=drawn for Greg; 0=drawn for Heather)	
NUMBER_DRAWN:	2	
C_COMMENT:	2 cpt tubes-first time draw	

Hit Update button

6.5. Update PTS Tracking. Enter Participant ID. Go to Cell Tab. Enter the following information for redraws:

CODE 3:	0	
App Date:	10/11/2006	MM/DD/YYYY
SAMP_OB:	5 (5=drawn for Greg; 0=drawn for Heather)	
NUMBER_DRAWN:	2 (add your 2 tubes to # already there)	
C_COMMENT:	2 cpt tubes- redraw on 10/11/06	

Hit update button

(Version: 06.10.16)

12.0 Urine Pregnancy Testing

Principle
To detect hCG in urine which is an indication of pregnancy.

MATERIALS

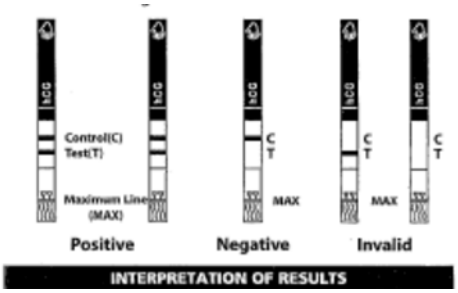
MooreBrand® hCG Dipstick	(Catalog No. 82789)

1. INFORMATION
   1.1. Record the receipt of test strips in the Reagent Inventory Book. Write number of kits received, lot number, date received, expiration date, and date kits put into use. Each kit contains 25 individually packaged strips.
   1.2. Test kits are stable until the expiration date on the carton and packages, when stored at room temperature (15-30 C).

2. METHOD

   2.1. Bring the urine specimen to room temperature before use. Mix the sample by gently swirling the container. Aliquot approximately 1 ml of urine into a 5 ml tube. Cloudy urine should be centrifuged or allowed to settle to obtain a clear specimen.
   2.2. Remove test strip from package.
   2.3. Set a timer for four minutes and 5 seconds. Hold the test strip at the top, in a vertical position with the arrows pointing down. Lower the test strip into the urine specimen and leave immersed for 5 seconds. Remove the strip from the urine and place it on a non-absorbent flat surface. Do not touch or jar the strip while the test is in progress.
   2.4. Read the results at four minutes.

3. INTERPRETATION



POSITIVE – TWO LINES indicates the presence of hCG.
NEGATIVE – ONE LINE indicates the absence of hCG.

4. QUALITY CONTROL
   4.1. Test strips are designed with a “control zone”. This control zone appears as a line at the top of the testing area of the strip, if the test is performed correctly. Absence of this line indicates incorrect performance or deterioration of reagents. Repeat the test with a new strip. If the control line still does not appear, open a new package.
   4.2. A positive and negative control is run weekly, at lot changes, and when a new kit is opened.

5. NOTIFICATION

5.1. The Research Center staff will inform the laboratory when there is a participant in-house who requires a pregnancy test. An appropriate form is prepared and a designated person from the lab will perform the test and return the results to the Research Center. The lab is required to fill out questions 3, 4 and 5 of the form and return to the Research Center.

6. REPORTING

6.1. Use a participant label to log sample onto the pregnancy testing log (**WS.031.PREGWS**). Complete the log sheet by checking results as positive or negative and fill in your initials. If a positive result occurs it must be confirmed by another staff member.

6.2. In the event of a positive result; do not pass this information on to the participant. The examining physician will inform the participant.

7. SAMPLE STABILITY

7.1. If testing is to be delayed more than a few hours, the urine can be stored at 2-8^{o}C for up to 48 hours.

8. CERTIFICATION OF STAFF

8.1. All staff who perform pregnancy testing, whether in lab or off-site, must be certified by the lab supervisor.

8.2. Training process includes:

- Review of the written protocol
- Review of the kit package insert
- Receipt of copies of both documents
- Certification form signed by the trainee and lab supervisor
- Records of all signed certifications are maintained by the lab supervisor

(Version: 03.21.16)

13.0 Current Cycle Testing

1. The table below indicates the laboratory tests performed on current cycle study groups.
2. Detailed instructions for each test are available in the Standard Operating Procedure – Analytical Book.

Test	Sample Type
Albumin	**Serum**
ALT	**Serum**
AST	**Serum**
Cholesterol	**Plasma**
Creatinine	**Serum**
CRP	**Serum**
Glucose	**Plasma**
HbA1c	**Whole Blood**
HDL	**Plasma**
Triglycerides	**Plasma**

(Version: 08.04.17)

14.0 Dried Blood Spot Protocol

PRINCIPLE/PURPOSE
Dried blood spots can be preserved for long periods with little deterioration of analytes.

MATERIALS

Materials	Order Number	Manufacturer/Vendor
Hirudin Blood Tube	08128812-001	Roche Diagnostics
Protein Saver Card	05715121	Fisher Scientific
Desiccant Packs	MSPP50P	Delta Adsorbents
Humidity Indicator Card	HIC1040C	Delta Adsorbents
Gas Impermeable Bag	0980016	Fisher Scientific

3. SPECIMEN COLLECTION/ PROCEDURE

1. Hirudin tube is drawn during the fasting phlebotomy (See SOP.Phlebotomy Protocol)
2. The tube is first used for platelet aggregation.
    - Tube has to sit for at least half an hour after being drawn. It is inverted 6 times before 110*u*l is aliquoted for Flow Cytometry. Another 320ul is aliquoted for Multiplate machine. Test takes 9 minutes. It is inverted again before 320*u*l is aliquoted for T-TAS machine. Test takes 10 minutes.
3. Any remaining sample is used for the blood spot
4. Place participant label DBS worksheet and on protein saver card.
5. Gently invert tube 2-4 times. Aspirate 50*u*l of whole blood using a pipette with a disposable tip. Transfer blood to the center of the first circle without touching the tip to the paper, saturating the circle fully. Repeat for the next 4 circles.
6. Allow the blood spots to dry horizontally on a clean paper towel for at least 4 hours at room temperature. Avoid direct sunlight and external heat sources.

4. BLOOD SPOT STORAGE

1. After blood spot is dry, fold flap down. Place DBS into a labelled gas impermeable bag.
2. Add humidity card and 3 desiccant packets.
3. Close bag and store in plastic container at -20^{o}C.
4. The protein saver card is not useable if the humidity indicator card turns pink.

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