LIPID-ATHEROSCLEROSIS LABORATORY

SERVICES GUIDE

For questions concerning protocol and assay development, validation of results, etc., please contact:

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GENERAL INFORMATION

INTRODUCTION

The intention of this manual is to provide guidelines for specimen collection and handling, as well as providing a guide of services currently available in the Lipid-Atherosclerosis Laboratory. The Lipid-Atherosclerosis Laboratory primarily performs specialized lipid and lipoprotein assays. In addition, other tests such as Lipoprotein(a), hs-CRP, Apolipoproteins B, A1, and C II, DNA Genotyping for Apo E, and Sterol Characterization (7-Dehydrocholesterol, Cholestanol, Beta-sitosterol) are available. To request lab tests, see section for specimen collection, handling, and storage. To request routine lab testing such as hematology or chemistry, see the Directory of Clinical Pathology Laboratory Services for proper specimen guidelines.

The tests listed in this Lipid-Atherosclerosis Laboratory Directory are only those which are currently performed or have been performed in the past. It is likely that additional new assays will be developed. Please contact the Laboratory Director or Laboratory Supervisors for test availability and scheduling.

LIPID-ATHEROSCLEROSIS LAB MISSION

The mission of the Lipid-Atherosclerosis Laboratory is to:

1. Perform analyses of lipids and lipoproteins that are not routinely available in the general hospital clinical laboratory or elsewhere in Oregon. For Sterols we are the only laboratory available on the West Coast.

2. Make laboratory facilities for the precise determination of lipids and lipoproteins available to all clinicians practicing at OHSU and to those in the surrounding area.

3. Perform specialized assays in the area of lipid metabolism that can be done with greater reliability than in other labs.

4. Develop new assays for clinical research which have the potential for wider applicability in diagnosis and patient care.
LIPID-ATHEROSCLEROSIS LAB HOURS OF OPERATION

The hours of operation of the Lipid-Atherosclerosis Laboratory are 9:30 a.m. to 5:30 p.m. Monday through Friday. **For blood drawn at night or on weekends, it is important to read section entitled "Specimen Collection" and "Specimen Labeling"**
SPECIMEN LABELING

Lab Request Slip:

The Lab Request Slip, as well as the samples, must be labeled when collected with the following information:

**Patient Name, ordering Physician’s name**

**Medical Record Number, DOB; Sex**

**Date and Time Drawn**  **NOTE:** If patient has not fasted for 10-12 hours, please write "non-fasting" on lab request slip.

**Research Samples:** Include Protocol # and PI – Deliver blood directly to MRB 421.

**Test to be performed**  **NOTE:** Computer ordering: (Out Patient & In Patient)

Currently, we are a reference lab to Clin. Path. Only a few tests will be done by the Lipid-Atherosclerosis Lab: Plasma Sterols (7-Dehydro cholesterol, Cholestanol & Beta-sitosterol); Chylomicron’s on pleural or other body fluid.  **For full Lipid Lab services blood must be directly delivered to the Lipid Lab and not go through Clin. Path.**

**24 hour urine - please include Total Volume**

**NOTE:** Any oral requests for lab testing must be followed by a written lab request document faxed or mailed within 5 days.

For referral samples sent to lab, this additional information is required:

- **Physician's full name and address** (if urgent include phone and fax numbers)
- **Billing information:** unless the patient has a current OHSU Account #, we will need to invoice the referring lab or physician – give address and phone #.
- **HIPAA Information:** Diagnosis Codes.

**SHIPPING ADDRESS:**

OHSU Lipid Lab, L465
MRB Room 421, Dock 4
3181 SW Sam Jackson Pk Rd
Portland, OR  97239-3098

**NOTE:** Ship samples Monday through Thursday by overnight express so they will be received within 24 hours of blood draw or follow directions for storage. Call lab at (503) 494-2005, 494-2008 or 494-4593 to notify us of shipment.
SPECIMEN COLLECTION

PLEASE READ CAREFULLY

Samples received at the Lipid-Atherosclerosis Lab will be spun, separated and stored in a 4°C, -20°C, or -70°C freezer by lab personnel for analysis or later referral.

Samples drawn after hours should be refrigerated at 4°C and delivered the next working day to Medical Research Building, Room 421.

EXCEPTION: Plasma for Apolipoprotein A1 and B, must be frozen at -70°C within 48 hrs of blood draw.

NOTE: All blood samples are to be transported in appropriate biohazard sealed containers that are leak proof.

UNACCEPTABLE SPECIMENS (Including, but not limited to, the following):

1. Specimens consisting of citrate, oxalate, or fluoride plasma (blue, gray, or black topped tubes) cannot be analyzed. (Preferred = lavender, green; red tops)
2. Specimens without the patients' name or collection date.
3. Specimens that were drawn more than 3 days before being received by the laboratory.
4. Specimens of less than 1 ml volume.
5. Specimens that include broken tubes.
6. Specimens not refrigerated or frozen as required under Specimen Handling.
7. Specimens for Apo E Genotyping cannot be clotted.
8. Specimens for Sterols cannot be grossly hemolyzed or < 0.5 ml of plasma.

This is not intended to imply that all "unacceptable" specimens should be discarded or not analyzed. Requesting physicians who send unacceptable specimens will be notified no later than the next working day. Also, the phlebotomist, if known, will be notified of the problem.
ASSAY PERFORMANCE AND RELEASE OF RESULTS

Assays performed by the Lipid-Atherosclerosis Lab are run on a batch basis whenever possible. Therefore, turnaround times will vary depending on which assay is requested. Turnaround time for lipids; HDL (Lipid Lab Panel) is 1-3 days. Turnaround time for beta-quant; chylo beta-quant is 3-7 days. Turnaround time for High Sensitivity CRP and Lp(a) is 1-3 days. Turnaround time for Apo A1* and Apo B* is 4-6 weeks. Turnaround time for DNA Genotyping for Apo E* is 4-6 weeks. Turnaround time for Sterols is 1-2 weeks. In the event lipids cannot be reported in 1-3 days requesting physicians will be notified. The Lipid-Atherosclerosis Director or Clinical Consultant reviews all Results. If samples are referred to our lab by Clin. Path., the final reports are faxed to Clin. Path. Lipid Clinic, Endocrinology Clinics and some research studies are resulted into Epic and sent to the physician. Reports that cannot be directly put into Epic may be taken to HIS Imaging for scanning. Most Research results are sent to the PI or study coordinator - they are not sent to Medical Records.

*Apo A1, Apo B, Apo C II; Apo E Genotyping are shipped out to Univ. of Washington – Northwest Lipid Research Lab in batches.

SAMPLE DISPOSAL

The Lipid-Atherosclerosis Lab is faced with space limitations, therefore samples will have only short term storage.
ROUTINE BIOCHEMICAL ASSAY BATCHING

The following tests are batched and analyzed by the Lipid-Atherosclerosis Lab or are referred via the Lipid Lab for analysis:

TEST

Cholesterol
Triglycerides
Chylos
VLDL*
LDL*
HDL*
Non-HDL-Cholesterol *
High Sensitivity CRP
LDL subfractions &/or HDL 2/3 **
LDL Density **
Apolipoprotein B **
Apolipoprotein A1 **
Apolipoprotein C II **
DNA Genotyping: Apolipoprotein E **
Lipoprotein (a)
Mevalonic Acid (plasma or urine) ****
Sterols (plasma – includes 7-Dehydrocholesterol, Cholestanol; Beta-sitosterol )

* Lipoproteins are analyzed by enzymatic methods which determine Cholesterol, Triglycerides, HDL and calculated VLDL Cholesterol, LDL Cholesterol and Non-HDL-Cholesterol. In samples where plasma Triglycerides exceed 300 mg/dl, lipoproteins are separated by preparative ultracentrifugation before analysis.

** Apo A1, Apo B, Apo C II, Apo E Genotyping, HDL 2/3; LDL Density are shipped out to Univ. of Washington Northwest Lipid Research Lab in Seattle, Washington.

**** Mevalonic Acid is available for research purposes only
COLOR CODING OF BLOOD COLLECTION TUBES

<table>
<thead>
<tr>
<th>TUBE</th>
<th>ADDITIVE</th>
<th>GENERAL USE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lavender top</td>
<td>EDTA(K2) Spray Dried 7 or 10 ml</td>
<td>Plasma</td>
</tr>
<tr>
<td>Green top</td>
<td>143 IU Sodium Heparin/5 or 10 ml</td>
<td>Plasma</td>
</tr>
<tr>
<td>Red top</td>
<td>none</td>
<td>Serum</td>
</tr>
</tbody>
</table>

*NOTE: EDTA is preferred.

SPECIMEN REQUIREMENTS FOR COMMON TESTS REQUESTED
(Please read "Specimen Collection on page 7")

<table>
<thead>
<tr>
<th>Test</th>
<th>Measures</th>
<th>Tube Needed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Lipids*</td>
<td>Cholesterol</td>
<td>7-10 ml lavender, green or red top</td>
</tr>
<tr>
<td></td>
<td>Triglycerides</td>
<td></td>
</tr>
<tr>
<td>2. Lipids/HDL*</td>
<td>Cholesterol</td>
<td>7-10 ml lavender, green or red top</td>
</tr>
<tr>
<td></td>
<td>Triglycerides</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calc. VLDL cholesterol</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calc. LDL cholesterol</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HDL cholesterol</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calc. Non-HDL-Cholesterol</td>
<td></td>
</tr>
<tr>
<td>3. Beta-quantification*</td>
<td>Cholesterol</td>
<td>7-10 ml lavender, green or red top</td>
</tr>
<tr>
<td></td>
<td>Triglycerides</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calc. LDL cholesterol</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HDL cholesterol</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VLDL cholesterol &amp; triglycerides</td>
<td></td>
</tr>
<tr>
<td>4. Ultracentrifugation*</td>
<td>Cholesterol</td>
<td>7-10 ml lavender, green or red top</td>
</tr>
<tr>
<td></td>
<td>Triglycerides</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calc.LDL cholesterol</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HDL cholesterol</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VLDL cholesterol &amp; triglycerides</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chylomicron cholesterol &amp; triglycerides</td>
<td></td>
</tr>
<tr>
<td>5. Apolipoprotein B*</td>
<td>LDL protein</td>
<td>7-10 ml lavender top*</td>
</tr>
</tbody>
</table>

*Note: One 7-10ml lavender or red top is sufficient for Chol, Trig, HDL, BQ/CBQ. If plasma triglycerides exceed 300 mg/dl, betaquantification will be done. If plasma triglycerides exceed 1000 mg/dl, ultracentrifugation for chylomicrons will be done.
<table>
<thead>
<tr>
<th></th>
<th>Test Name</th>
<th>Description</th>
<th>Volume and Top</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Apolipoprotein A1*</td>
<td>HDL protein</td>
<td>7-10 ml lavender top*</td>
</tr>
<tr>
<td>7</td>
<td>Apolipoprotein CII*</td>
<td>VLDL protein</td>
<td>7-10 ml lavender top*</td>
</tr>
<tr>
<td>8</td>
<td>High Sensitivity CRP</td>
<td>C-Reactive Protein</td>
<td>7-10 ml lavender, green or red top</td>
</tr>
<tr>
<td>9</td>
<td>HDL 2/3</td>
<td>HDL Subfractions 2&amp;3</td>
<td>10 ml lavender top</td>
</tr>
<tr>
<td>10</td>
<td>LDL Density</td>
<td>LDL Density- Relative Flotation</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Lipoprotein(a)</td>
<td>Lp(a)</td>
<td>7-10 ml lavender, green or red top*</td>
</tr>
<tr>
<td>12</td>
<td>DNA Genotyping: Apo E</td>
<td>Presence of specific alleles</td>
<td>10 ml lavender top</td>
</tr>
<tr>
<td>13</td>
<td>Plasma Sterols</td>
<td>7-Dehydrocholesterol, cholestanol, sitosterol, desmosterol and lathosterol</td>
<td>3 ml lavender or red top or green top</td>
</tr>
<tr>
<td>14</td>
<td>Plasma mevalonic acid</td>
<td>Mevalonic acid</td>
<td>10 ml green top</td>
</tr>
<tr>
<td>15</td>
<td>Urinary mevalonic acid</td>
<td>Mevalonic acid</td>
<td>2 x 10 ml urine</td>
</tr>
</tbody>
</table>

*Note: Must reach lab within 48 hrs or be spun, poured off and frozen within 48 hours
REFERRAL SERVICE

The Lipid Lab sends out Apo A1, Apo B, Apo C II, Apo E Genotyping, HDL 2/3; LDL Density to the University of Washington Northwest Lipid Research Lab in Seattle, Washington. The Lipid Lab can also refer samples for analysis to other lipid research laboratories in the United States. This is generally for research purposes. Please contact Lab Supervisor for more information.

REFERENCE RANGES

<table>
<thead>
<tr>
<th>Test</th>
<th>Units</th>
<th>MALE 0-18</th>
<th>MALE 18-45</th>
<th>MALE 45+</th>
<th>FEMALE 0-18</th>
<th>FEMALE 18-45</th>
<th>FEMALE 45+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>mg/dl</td>
<td>&lt;180</td>
<td>&lt;200</td>
<td>&lt;220</td>
<td>&lt;180</td>
<td>&lt;200</td>
<td>&lt;220</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>mg/dl</td>
<td>&lt;90</td>
<td>&lt;150</td>
<td>&lt;150</td>
<td>&lt;90</td>
<td>&lt;150</td>
<td>&lt;150</td>
</tr>
<tr>
<td>LDL</td>
<td>mg/dl</td>
<td>&lt;100</td>
<td>&lt;100</td>
<td>&lt;100</td>
<td>&lt;100</td>
<td>&lt;100</td>
<td>&lt;100</td>
</tr>
<tr>
<td>HDL</td>
<td>mg/dl</td>
<td>&gt;45</td>
<td>&gt;45</td>
<td>&gt;45</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>mg/dl</td>
<td>&lt;30</td>
<td>&lt;30</td>
<td>&lt;30</td>
<td>&lt;30</td>
<td>&lt;30</td>
<td>&lt;30</td>
</tr>
</tbody>
</table>

NOTE: For adults, the NCEP ATP III recommends mg/dl as follows:

**Total Cholesterol:** Desirable: <200 Borderline high: 200-239 High: >240

**LDL Cholesterol:** Optimal: <100 Above optimal: 100-129 Borderline high: 130-159 High: >160-189 Very high: > 190 If severe CHD present, LDL <70 = Goal.

**HDL Cholesterol:** Low: < 40 High: ≥ 60

**Triglycerides:** Normal: < 150 Borderline high: 150-199 High: 200-499 Very high: ≥ 500

Note: Certain drugs may alter the lipid profile.

VLDL-C mg/dl < 31

VLDL-C mg/dl Normal ratio <0.25 as determined by beta-quantification.

Total Trig. mg/dl Normal ratio >0.30 indicates type III hyperlipidemia.

Chylomicrons mg/dl None detectable in 12-16 hour fasting sample. Presence of chylomicrons along with plasma triglycerides >1000 can indicate types I or V hyperlipidemia.

Non-HDL-Chol < 130

**High Sensitivity CRP** mg/L Normal ≤ 2.0 High >2.0

**NOTE:** Reference ranges listed for the Lipid-Atherosclerosis Lab are based on NHLBI Guidelines; Surgeon General of USA.
Clinical Significance of Genotyping Results:

Apo E: In the presence of elevated triglycerides, E2/E2 genotype is diagnostic for type III hyperlipidemia. The Apo E4 allele is associated with elevated levels of plasma cholesterol.
**LIST OF AVAILABLE TESTS AND METHODOLOGY**

**CHOLESTEROL, TOTAL**

7-10 ml Lavender, green or Red Top plasma preferred/serum

(* Order as lipids)

**Method:** Cholesterol esterase, oxidase, and peroxidase as described by Allain and Trinder. Roche/Boehringer Mannheim Corp. High Performance Cholesterol Reagent; Hitachi 704 Chemistry Analyzer. Specificity is enzymatic with 99.5% hydrolysis of all Cholesterol esters. Linear up to 400 mg/dl without dilution. Hemolysis up to 200 mg/dl shows no interference. Cholesterol is stable up to 6 days at 2-8°C.

**Precision:** In-house inter-assay CV < 2.0%

**Reference Ranges:** See page 12 for values.

Assay is batched by the Lipid-Atherosclerosis Lab in groups of 24 or more and analyzed Tuesday through Friday (except holidays).

**TRIGLYCERIDES, TOTAL**

7-10 ml Lavender, green or Red Top plasma preferred/serum

(* Order as lipids)

**Method:** Triglyceride hydrolysis by microbial lipase, the glycerol is then oxidized to dihydroxyacetene phosphate and hydroxy peroxide - a modified version of Wahlefeld. Roche/Boehringer Mannheim Corp. Triglyceride/GPO Reagent; Hitachi 704 Chemistry Analyzer. Specificity is enzymatic for complete hydrolysis to free glycerol. Linear up to 500 mg/dl without dilution. Hemolysis up to 700 mg/dl shows no interference. Do not use Glycerol coated stoppers or vials. Triglycerides are stable up to 6 days at 2-8°C.

**Precision:** In-house inter-assay CV < 5.0%

**Reference Ranges:** See page 12 for values.

Assay is batched by the Lipid-Atherosclerosis Lab in groups of 24 or more and analyzed Tuesday through Friday (except Holidays).
**HDL CHOLESTEROL**

7-10 ml Lavender, green or Red Top plasma preferred/serum

(*) Order as Lipids; HDL

Method: Cholesterol and Triglyceride analysis by enzymatic determination as described previously. HDL by homogenous methodology and quantitative analysis with calculated VLDL and LDL as described by Bachorik, Albers; Warnick. *Note - If Plasma Triglycerides exceed 300 mg/dl a beta-quant will be done to directly measure the VLDL. Roche/BMC homogeneous reagent and Hitachi 704 Chemistry Analyzer.

**Precision:** In-house inter-assay CV < 3.5%

Reference Range: See page 12 for values.

Assay is batched by the Lipid-Atherosclerosis Lab in groups of 10 or more; analyzed Tuesday through Friday (except Holidays)

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**BETA-QUANTIFICATION**

7-10 ml Lavender, green or Red Top plasma preferred/serum

(*) Order as Lipids; Beta-quant

Method: Cholesterol and triglyceride analysis by enzymatic determination as described previously. Plus, ultracentrifugation methodology to isolate and quantitate VLDL, with calculated LDL and quantitated HDL. *Note - If the Plasma Triglycerides exceed 1000 mg/dl an ultracentrifugation for Chylomicrons will also be done. Beckman L8-80 Ultracentrifuge and Hitachi 704 Chemistry Analyzer.

Reference Ranges: See page 12 for values.

Assay is batched by the Lipid-Atherosclerosis Lab in groups of 2 or more for ultracentrifugation.

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**CHYLOMICRONS**

7-10 ml Lavender, green or Red Top plasma preferred/serum, occasionally done on Pleural Fluid/Chest Tube Drainage 3-5 ml of Pl. Fluid/C.T.Drain. (syringe or urine specimen container)

(*) Order as Chylomicrons; Beta-quant-for full profile

Method: chylomicrons are isolated by ultracentrifugation and analyzed for Cholesterol and Triglyceride content by enzymatic methodology. Beckman L8-80 Ultracentrifuge; Hitachi 704 Chemistry Analyzer.

Reference Ranges: See page 12 for values.

Assay is batched by the Lipid-Atherosclerosis Lab in groups of 2 or more for ultracentrifugation.
VLDL, LDL; HDL by FULL ULTRACENTRIFUGATION
10 ml Lavender Top
plasma preferred/serum

(* Order as Full Ultra)

Method: Ultracentrifugation spins at various densities of saline with KBR to isolate specific lipoprotein fractions as described by Havel. Enzymatic determination of cholesterol and triglyceride content of each fraction. Beckman L8-80 Ultracentrifuge; Hitachi 704 Chemistry Analyzer.

Reference Ranges: See page 12 for values. Expected ranges vary with age, genotype and hormonal status.

Assay is batched by the Lipid-Atherosclerosis Lab in groups of 2 or more for ultracentrifugation.

High Sensitivity CRP
7-10 ml Lavender, green or Red Top
Plasma preferred/serum
(* Order as high sensitivity CRP)

Method: Latex Immunoassay where the reagent is highly specific forming an insoluble complex. C-Reactive Protein is then measured quantitatively. Genzyme Diagnostics CRP-UWR kit and Hitachi 704 Chemistry Analyzer.

Precision: In-house interassay CV < 5.5%

Reference Ranges: See page 12 for values

Assay is batched by the Lipid-Atherosclerosis Lab in groups of 2 or more and analyzed Wednesday and Friday (except Holidays).
LIPOPROTEIN(a)  
5 ml Lavender, Green or Red Top 
plasma preferred/serum

**Method:** Immunoturbidimetric Methodology for quantitation of LP (a). Polymedco’s LP (a) test kit; Hitachi 704 Chemistry Analyzer.

**Precision:** In-house inter-assay CV < 5%

**Reference Ranges:** See page 12 for values or call ext. 4-2005 for this information.

Assay is batched by the Lipid Lab in groups of 2 or more and analyzed Wednesday and Friday (except Holidays).

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DNA Genotyping  
10 ml Lavender Top

White blood cells (buffy coat needed) 
Clotted blood is not usable. EDTA preferred as anticoagulant

**Methods:**
Apo E: PCR amplification. This test is sent out to Univ. of Washington – Northwest Lipid Research Lab in Seattle.
**STEROLS**

3 ml Lavender, Green or Red Top

Plasma preferred/serum (minimum 1.0 ml)

(* Order as Smith-Lemli-Opitz syndrome (SLOS), Cerebrotendinous xanthomatosis (CTX), Sitosterolemia or Plasma Sterols, Misc)

**METHOD:** For Clinical Patients: Quantitative determination for the presence or absence of 7-Dehydrocholesterol to rule out the Smith-Lemli-Opitz Syndrome, Cholestanol for CTX; Beta-sitosterol for Sitosterolemia.

**NOTE:** For information about Sterols please contact Dr. Louise Merkens, Sterols Lab Section Supervisor at extension 4-4593.

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**MEVALONIC ACID**

10 ml Green Top

Plasma preferred/ can also be done on urine

(Note: Test is for Research purposes – by special request only!)

For more information about Mevalonic Acid or to make a special request, please call extension 4-2007 and speak with Dr. Duell or call 4-4593 and speak with Dr. Louise Merkens.
LAB DIRECTOR’S REVIEW PAGE

The Lipid-Atherosclerosis Laboratory Services Guide was reviewed and any changes approved on the following date by the Lab Director.

_________________________________________   _______________________
Signature                                      Date