STEROL ANALYSIS LABORATORY

SERVICES GUIDE

Revised 6/2018
For questions concerning protocol and assay development, validation of results, quality control, sample handling, test results, please contact:

Paul Barton Duell, MD  
Director, Sterol Analysis Laboratory  
Phone: (503) 494-2007

Andrea DeBarber, PhD  
Supervisor, Sterols Analysis Laboratory  
Phone: (503) 494-4593
Table of Contents

General Information

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>4</td>
</tr>
<tr>
<td>Laboratory Mission</td>
<td>4</td>
</tr>
<tr>
<td>Laboratory Hours of Operation</td>
<td>4</td>
</tr>
<tr>
<td>Specimen Collection</td>
<td>5</td>
</tr>
<tr>
<td>Shipping Labeling and Shipping</td>
<td>6</td>
</tr>
<tr>
<td>Assay Timing and Release of Results</td>
<td>7</td>
</tr>
<tr>
<td>Sample Disposal</td>
<td>7</td>
</tr>
<tr>
<td>Assay Batching</td>
<td>7</td>
</tr>
<tr>
<td>Color Coding of Blood Collection Tubes</td>
<td>8</td>
</tr>
<tr>
<td>Specimen Requirements for Common Tests Requested</td>
<td>8</td>
</tr>
<tr>
<td>Referral Service</td>
<td>8</td>
</tr>
<tr>
<td>Reference Ranges</td>
<td>9, 10</td>
</tr>
</tbody>
</table>

List of Available Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Cholesterol</td>
<td>9</td>
</tr>
<tr>
<td>Plasma Sterols</td>
<td>9</td>
</tr>
<tr>
<td>Urinary Bile Alcohols</td>
<td>10</td>
</tr>
<tr>
<td>Plasma 7a12aC4</td>
<td>10</td>
</tr>
<tr>
<td>Dried Blood Spot 7a12aC4</td>
<td>10</td>
</tr>
</tbody>
</table>
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 offered by the Sterol Analysis Laboratory. The Sterol Analysis Laboratory primarily performs quantification of stanols/sterols and intermediates in the bile acid pathway. To request lab tests, see section for specimen collection, handling, and storage.

The tests listed in this Sterol Analysis 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 Supervisor for test availability and scheduling.

STEROL ANALYSIS LABORATORY MISSION

The mission of the Sterol Analysis Laboratory is to:

1. Perform analyses of stanols/sterols and intermediates in the bile acid pathway that are not routinely available in the general hospital clinical laboratory or elsewhere in Oregon. We are currently the only laboratory available on the West Coast to offer this type of testing.

2. Make laboratory services to measure stanols/sterols and intermediates in the bile acid pathway for diagnostic testing available to all clinicians practicing at OHSU and to those in the surrounding area.

3. Develop new assays for clinical research which have the potential for wider applicability in diagnosis and patient care.

STEROL ANALYSIS LABORATORY HOURS OF OPERATION

The hours of operation of the Sterol Analysis Laboratory are 9:00 a.m. to 5:00 p.m. Tuesday through Friday. For blood drawn at night or on weekends, it is important to read the sections entitled "Specimen Collection" and "Specimen Labeling".
SPECIMEN COLLECTION

PLEASE READ CAREFULLY

Blood samples drawn locally after hours should be refrigerated at 4°C and delivered the next working day to Richard Jones Hall, Room 3360 or shipped to the Sterol Analysis Laboratory using overnight delivery. Whole blood specimens should be shipped on ice (insulate sample and include one “ice pack”, do not freeze). Plasma should be shipped frozen. Urine can be shipped on ice or frozen. Dried blood spots can be shipped at room temperature.

Whole blood samples received by the Sterol Analysis Laboratory will be spun, separated and plasma will be analyzed immediately or frozen prior to analysis.

NOTE: All blood, plasma and urine 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.

2. Specimens without at least two identifiers (to include patients' name, DOB or MRN).

3. Blood/plasma specimens of less than 0.5 ml volume.

4. Specimens that include broken tubes.

5. Specimens not refrigerated or frozen as required.

6. Whole blood specimens drawn more than 3 days before receipt by the laboratory and that are grossly hemolyzed.

This is not intended to imply that all "unacceptable" specimens will 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.
SPECIMEN LABELING

Laboratory Sample Requisition Form:

The Samples must be labeled with at least two identifiers, including patient name, DOB, MRN. The Laboratory Sample Requisition Form submitted with samples must provide the following information:

- Patient Name
- Ordering Physician’s name
- Medical Record Number (MRN)
- DOB, Sex
- Date drawn
- Test to be performed

NOTE: Computer ordering: (Out-Patient & In-Patient)
Currently, we are a reference lab to OHSU Clinical Pathology. Only a few tests will be done by the Sterol Analysis Laboratory: plasma 7-Dehydrocholesterol, plasma 5α-cholestanol, plasma beta-sitosterol, and plasma sterols, misc. Also Urine, Bile alcohol (5β-cholestan-3α,7α,12 α,23S,25-pentol). Also dried blood spot or plasma 7α,12α-dihydroxy-4-cholesten-3-one.

For samples directly delivered or shipped to the Sterol Analysis Laboratory, this additional information is required:

- Physician’s full name and address (please include phone and email address)
- Billing information: we will need to invoice the referring laboratory or bill the patient directly – please provide address, phone number and email.

SHIPPING ADDRESS:
Attention: Andrea DeBarber (503-494-4593)
Physiology & Pharmacology Department (L334)
RJH Room 3360, Dock 4, Oregon Health & Science University
3181 SW Sam Jackson Park Road
Portland, OR 97239-3098

NOTE: Specimens should be shipped by overnight express carrier Monday through Thursday. Saturday delivery may be available upon request. Call Laboratory at (503) 494-4593 or email tracking number to debarber@ohsu.edu to notify us of shipment.
ASSAY TIMING AND RELEASE OF RESULTS

Assays performed by the Sterol Analysis Laboratory are run on a batch basis whenever possible. Therefore, turnaround times will vary depending on which assay is requested. Turnaround time for plasma Sterols and urine bile alcohols is 2-4 weeks. The Sterol Analysis Director or Laboratory Supervisor reviews all results.

If samples are referred to our Laboratory by Clinical Pathology, the final reports are sent to Clinical Pathology to be scanned into Beaker for reporting in Epic. For samples submitted directly to the Sterol Analysis Laboratory reports are sent to the requesting physician and referring laboratory. Research results are sent to the PI or study coordinator.

SAMPLE DISPOSAL

The Sterol Analysis Laboratory is faced with space limitations. Therefore, samples will only be stored for 2 years.

ASSAY BATCHING

The following tests are batched and analyzed by the Sterol Analysis Laboratory or are referred to the OHSU Lipid Laboratory for analysis:

Plasma cholesterol, total*
Plasma sterols (includes 7-dehydrocholesterol, 5α-cholestanol; Beta-sitosterol)
Urinary bile alcohol (5β-cholestan-3α,7α,12 α,23S,25-pentol)
Plasma or dried bloodspot bile acid pathway intermediate (7α,12α-dihydroxy-4-cholesten-3-one)

* Samples are sent out for total cholesterol testing to the OHSU Lipid-Atherosclerosis Laboratory.
# 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/2 or 3 ml</td>
<td>Plasma</td>
</tr>
<tr>
<td>Green top</td>
<td>143 IU Sodium Heparin/2 or 3 ml</td>
<td>Plasma</td>
</tr>
<tr>
<td>Red top</td>
<td>None</td>
<td>Serum</td>
</tr>
</tbody>
</table>

# SPECIMEN REQUIREMENTS FOR COMMON TESTS REQUESTED

(Please read "Specimen Collection" on page 5)

<table>
<thead>
<tr>
<th>Test</th>
<th>Measures</th>
<th>Tube Needed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Plasma Cholesterol*</td>
<td>Cholesterol, total</td>
<td>3 ml lavender, green or red top</td>
</tr>
<tr>
<td>2. Plasma Sterols*</td>
<td>7-Dehydrocholesterol, 5α-cholestanol or beta-sitosterol</td>
<td>3 ml lavender or green top</td>
</tr>
<tr>
<td>3. Plasma Bile Acid Pathway Intermediate*</td>
<td>7α,12α-Dihydroxy-4-cholesten-3-one</td>
<td>3 ml lavender or green top</td>
</tr>
<tr>
<td>4. Plasma Bile Acid Pathway Intermediate</td>
<td>7α,12α-Dihydroxy-4-cholesten-3-one</td>
<td>Capillary or venous blood spotted onto filter-paper and dried</td>
</tr>
<tr>
<td>5. Urinary Bile Alcohols</td>
<td>5β-Cholestane-3α,7α,12α,23S,25-pentol</td>
<td>10 ml random urine</td>
</tr>
</tbody>
</table>

*Note:* Whole blood specimens must reach laboratory within 48-72 hrs or be spun, poured off and plasma frozen within 48-72 hours.

# REFERRAL SERVICE

The Sterol Analysis Laboratory sends out samples for total cholesterol testing to the OHSU Lipid Atherosclerosis Laboratory. The Sterol Analysis may also refer samples for analysis to other lipid research laboratories in the United States. This is generally for research purposes. Please contact Laboratory Director for more information.
LIPID LABORATORY CHOLESTEROL REFERENCE RANGES

Reference Ranges:

0-18 years <180 mg/dL (both male & female)
18-45 years <200
>45 years <220

Note – For adults, the NHLBI ATP III recommends the following: Cholesterol

Desirable <200 mg/dL
Borderline High 200-239
High >240

Note: Certain drugs may alter the lipid profile.

NOTE: Reference ranges listed are based on NHLBI Guidelines; Surgeon General of USA.

LIST OF AVAILABLE TESTS AND METHODOLOGY

OFFERED THROUGH THE LIPID LABORATORY:

CHOLESTEROL, TOTAL

3 ml Lavender, Green or Red Top
Plasma preferred/serum

Method: The cholesterol assay uses an enzymatic, colorimetric process. Cholesterol esters are
 cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. Cholesterol
 oxidase then catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide.
 In the presence of peroxidase, the hydrogen peroxide formed effects the oxidative coupling of
 phenol and 4-aminophenazone to form a red quinone-imine dye. The color intensity of the dye
 formed is directly proportional to the cholesterol concentration. It is determined by measuring
 the increase in absorbance. Linear up to 800 mg/dL without dilution. No significant interference
 from Hemolysis up to 700 mg/dL. Cholesterol is stable up to 7 days at 2-8°C.

Precision: In-house inter-assay CV < 2.0%
Reference Ranges: See page 10 for values

Assay is analyzed by the Lipid Laboratory Tuesday through Friday (except holidays).

OFFERED THROUGH THE STEROL ANALYSIS LABORATORY:

PLASMA STEROLS

3 ml Lavender, Green or Red Top
EDTA (Lavender) plasma preferred
(minimum 1.0 ml)

Precision: In-house inter-assay CV < 15%
Reference Ranges: Call 503-494-4593 for this information.

Assay is batched by the Sterol Analysis Lab in groups of 4 or more samples and analyzed at least once every two weeks.

<table>
<thead>
<tr>
<th>URINE BILE ALCOHOL</th>
<th>5 ml Random Urine, no preservatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>5β-CHOLESTANE-3α,7α,12α,23S,25-PENTOL</td>
<td>(minimum 2.0 ml)</td>
</tr>
</tbody>
</table>

Method: Addition of 23S-pentol-d6 method internal standard followed by incubation of urine with beta-glucuronidase enzyme. Isotope-dilution quantification is performed using negative-mode LC-ESI-MS/MS multiple reaction monitoring of 23S-pentol (m/z 451>357) and internal standard (m/z 457>357) with a reversed-phase gradient utilizing a 2.1x50mm Biphenyl (2.6 μm) column.


Precision: In-house inter-assay CV < 15%
Reference Ranges: Call 503-494-4593 for this information.

Assay is batched by the Sterol Analysis Lab in groups of 4 or more samples and analyzed at least once every two weeks.

<table>
<thead>
<tr>
<th>PLASMA</th>
<th>3 ml Lavender, Green or Red Top EDTA (Lavender) plasma preferred</th>
</tr>
</thead>
<tbody>
<tr>
<td>7α,12α-DIHYDROXY-4-CHOLESTEN-3-ONE</td>
<td>(minimum 1.0 ml)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DRIED BLOODSPOT</th>
<th>Capillary or venous blood collected using Whatman 903 filter-Paper (Guthrie card)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7α,12α-DIHYDROXY-4-CHOLESTEN-3-ONE</td>
<td></td>
</tr>
</tbody>
</table>

Method: Method: Addition of 7alpha,12alpha-dihydroxy-4-cholesten-3-one-d9 method internal standard in methanol to sample and derivatization as previously described. Isotope-dilution quantification is performed using positive-mode LC-ESI-MS/MS multiple reaction monitoring of 7alpha12alphaC4 (m/z 531.7>152.1) and internal standard (m/z 440.7>152.1) with a reversed-phase gradient utilizing a 2.1x50mm Biphenyl (2.6 μm) column.


Precision: In-house inter-assay CV < 15%
Reference Ranges: Call 503-494-4593 for this information.

Assay is batched by the Sterol Analysis Lab in groups of 4 or more samples and analyzed at least once every two weeks.
The Sterol Analysis Laboratory Services Guide was reviewed and any changes approved on the following date by the Laboratory Director.

Signature [Signature]

Date [6/8/18]