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Laboratory Phone: 503-494-4593

CAP # 2442607 **CLIA # 38D06-56829**

Title: Sterol Analysis Clinical Laboratory Services Guide

STEROL ANALYSIS CLINICAL LABORATORY SERVICES GUIDE

For questions concerning laboratory protocols, assay development and validation, quality control, sample handling and assay results, please contact:

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

Andrea E. DeBarber, PhD
Technical Supervisor, Sterol Analysis Laboratory
Phone: (503) 494-4593

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GENERAL INFORMATION

INTRODUCTION

The intention of this manual is to provide guidelines for specimen collection and handling, as well as provide 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 Service Guide are only those which are currently performed or have been performed in the past. It is likely that 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 general hospital clinical laboratories or elsewhere in Oregon. We are currently the only laboratory 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 8:00 a.m. to 5:00 p.m. Monday through Friday. **For blood drawn at night or on weekends, it is important to read the sections entitled "Specimen Collection" and "Specimen Labeling, Ordering Tests and Shipping".**

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 packs (insulate sample and include one ice-pack, do not freeze). Plasma should be shipped on ice packs or frozen. Urine should be shipped on ice-packs or frozen. Whole blood samples received by the Sterol Analysis Laboratory will be spun and separated. Plasma will be analyzed immediately or frozen prior to analysis.

NOTE: All blood, plasma and urine samples should 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).
2. Specimens without at least two identifiers (i.e. patient name, DOB and/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 one day before receipt by the laboratory and/or that are grossly hemolyzed.
7. Specimens that contain any needles or sharps.

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. The phlebotomist, if known, will be notified of the problem.

SPECIMEN LABELING, ORDERING TESTS AND SHIPPING

Samples must be labeled with at least two identifiers, i.e. patient name, DOB and/or MRN.

NOTE: ordering using EPIC (OHSU Out-Patient & In-Patient). Currently, we are a reference lab to OHSU Clinical Pathology. Tests performed by the Sterol Analysis Laboratory for OHSU include: plasma 7-dehydrocholesterol, plasma 5α–cholestanol, plasma β-sitosterol, plasma sterols, misc. and urine bile alcohol (5β-cholestane-3α,7α,12 α,23S,25-pentol).

The Laboratory Sample Requisition Form (or EPIC order) associated with the sample must provide the following information:

- **Patient Name**
- **Ordering Physician's name**
- **Medical Record Number (MRN)**
- **DOB, Sex**
- **Date of specimen collection**
- **Test to be performed**

For samples shipped directly to the Sterol Analysis Laboratory from outside OHSU, please include:

- **A paper Sample Requisition Form, completed and signed by Ordering Physician**
- **We will need to invoice the referring laboratory or bill the patient directly – if patient is responsible, please provide patient address, phone number and email.**

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 lab of shipment.

SHIPPING ADDRESS:
Attention: Andrea DeBarber (503-494-4593)
Richard Jones Hall Room 3340/3360, Dock 4, Oregon Health & Science University
3181 SW Sam Jackson Park Road
Portland, OR 97239-3098

ASSAY TIMING AND RELEASE OF RESULTS

Assays performed by the Sterol Analysis Laboratory are analyzed on a batch basis whenever possible. Therefore, turnaround times will vary depending on which assay is requested. Turnaround time for each assay is provided in Laboratory Services Guide. The Sterol Analysis Director or Technical Supervisor/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 and reported in EPIC. For samples submitted directly to the Sterol Analysis Laboratory reports are emailed and/or Faxed to the requesting physician and/or 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 are generally not stored for longer than 2 years.

ASSAY BATCHING

The following tests are batched and analyzed by the Sterol Analysis Laboratory for analysis:

Plasma/serum sterols (includes 7-dehydrocholesterol, 5α-cholestanol; β-sitosterol)
Plasma/serum bile acid pathway intermediates (7α,12α-dihydroxy-4-cholesten-3-one and 7α-hydroxy-4-cholesten-3-one)
Plasma/serum bile alcohol (5β-cholestane-3α,7α,12α,25-tetrol-3-O-β-D-glucuronide)
Urinary bile alcohol (5β-cholestane-3α,7α,12 α,23S,25-pentol)

Prior to use for patient testing the laboratory requires that all consumable material used in testing meets manufacturers or laboratory specifications.

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COLOR CODING OF BLOOD COLLECTION TUBES

TUBE	ADDITIVE	GENERAL USE
Lavender top	EDTA(K2) Spray Dried/2 or 3 mL	Plasma
Green top	143 IU Sodium Heparin/2 or 3 mL	Plasma
Red top	None	Serum

SPECIMEN REQUIREMENTS FOR COMMON TESTS REQUESTED

(Please read also "Specimen Collection" on page 3)

TEST	MEASURE	TUBE REQUIRED
1. Plasma/serum sterol[1]	7-Dehydrocholesterol, total	3 mL lavender or green top (plasma) or 3 mL red top (serum
2. Plasma/serum sterol[1]	5α-Cholestanol, total	3 mL lavender or green top (plasma) or 3 mL red top (serum
3. Plasma/serum sterol[1]	β-sitosterol, total	3 mL lavender or green top (plasma) or 3 mL red top (serum
4. Plasma/serum bile acid pathway intermediate[1]	7α,12α-Dihydroxy-4-cholesten-3-one, free	3 mL lavender or green top (plasma) or 3 mL red top (serum
5. Plasma/serum bile acid pathway intermediate[1]	7α-Hydroxy-4-cholesten-3-one, free	3 mL lavender or green top (plasma) or 3 mL red top (serum
6. Plasma/serum bile alcohol[1]	5β-cholestane-3α,7α,12α,25-tetrol-3-O-β-D-glucuronide	3 mL lavender or green top (plasma) or 3 mL red top (serum
7. Urinary bile alcohol	5β-Cholestane-3α,7α,12α, 23S,25-pentol, total	10 mL random urine

1. **Note:** Whole blood can be shipped on ice-packs (not frozen). Whole blood specimens must reach laboratory within 2-4 days of collection.

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LIST OF AVAILABLE TESTS AND METHODOLOGY

OFFERED THROUGH THE STEROL ANALYSIS CLINICAL LABORATORY:

PLASMA/SERUM STEROLS, TOTAL

Plasma (preferred)/serum	3 mL lavender or green top (plasma) 3 mL red top (serum) (minimum volume 1 mL)

Method: Hexane extraction of saponified plasma lipids followed by derivatization. Gas chromatography for quantitative determination of elevated cholestanol (for CTX); 7-dehydrocholesterol (for diagnosis of Smith-Lemli-Opitz Syndrome), β-sitosterol (for Sitosterolemia). Quantification is by flame ionization detection or selected-ion monitoring, ion-ratio fragmentometry in the electron impact mode using epicoprostanol as internal standard. Linear up to 4.8 mg/dL without dilution.

References: Kelley RI (1995) Clin Chim Acta 236:45, Merkens LS et al (2009) J Pediatr 154:557.

Precision: In-house inter-assay CV < 15%

Sample Stability: Plasma/serum is stable up to 17 hours at ambient temperature or refrigerated. Samples can be frozen for long-term storage. Plasma can be shipped overnight on ice-packs or frozen. Whole blood can be shipped overnight on ice-packs (not frozen).

Reference Ranges:

Normal cholestanol[1,2] concentration in unaffected individuals age range 0.2-18 years old, n= 330 and 18-70 years old, n=39	Pediatric (0.2-18 years old): 0.27 ± 0.08 mg/dL [0.10-0.65] Adult (18-70 years old): 0.25 ± 0.07 mg/dL [0.11-0.44] **mean ± SD [range]**
Cholestanol concentration[3] in CTX-affected untreated individuals age range 5.2-33 years old, n=14	3.01 ± 1.40 mg/dL [1.31-7.10] **mean ± SD [range]**
Normal 7-Dehydrocholesterol concentration in children age range 0.1-16 years old, n=153	< 0.10 mg/dL
Normal sitosterol[4] concentration in unaffected individuals age range 0.2-17 years old, n= 330 and 18-70 years old, n=39	Pediatric (0.2-17 years old): 0.31 ± 0.14 mg/dL [0.03-0.94] Adult (18-70 years old): 0.24 ± 0.14 mg/dL [0.09-0.67] **mean ± SD [range]**

1. *Note that medications such as Prednisone, Ezetimibe, and statins may result in normal blood/plasma cholestanol, leading to false negative result for CTX. (Siman-Tov T et al (2006) J Neurol Sci. **243(1-2)**:83–86, DeBarber AE et al (2024) J Clin Lipidol.**18(3)**:e465-e476.).*
2. *Note the 99th percentile concentration of cholestanol was 5.4 mg/L (Schaefer et al (2016) Endotext).*
3. *Note that cholestanol may also be >1.00 mg/dL for other conditions, for example in cholestasis and liver disease (Koopman BJ et al (1984) Clin Chem Acta **137(3)**:305-315).*
4. *99th percentile concentration of β-sitosterol previously reported as 7.5 mg/L (Schaefer et al (2016) Endotext.).*

Assay is batched by the Sterol Analysis Laboratory with a resultant turn-around time for reporting of 20 business days.

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PLASMA/SERUM BILE ACID PATHWAY INTERMEDIATE

7α,12α-DIHYDROXY-4-CHOLESTEN-3-ONE, FREE

Plasma (preferred)/serum	3 mL lavender or green top (plasma) 3 mL red top (serum) (minimum volume 0.5 mL)

Method: Addition of 7α,12α-dihydroxy-4-cholesten-3-one-d_7 method internal standard in methanol to sample and extracted as previously described. Isotope-dilution quantification is performed using positive-mode LC-ESI-MS/MS multiple reaction monitoring of 7α12αC4 (m/z 417.3>253.1 and 417.3>381.2) and internal standard (m/z 424.3>253.1) with a reversed-phase gradient utilizing a 150 x 4.6 mm Biphenyl (2.6 µm) column. Linear up to 1,000 ng/mL without dilution. No significant interference from common over the counter and prescription medications. Carry over response in blanks is < 6% of the LLOQ for the analyte and < 1% for the internal standard.

References: DeBarber AE et al (2018) J Lipid Res. 59 (11):2214-2222, DeBarber AE et al (2014) J Lipid Res. 55(1):146-54, DeBarber AE et al (2014) Clin Biochem. 47(9):860-3.

Precision: In-house intra-assay CV < 7% for low QC n=20 samples and < 7% for high QC n=20 samples

Accuracy: In-house intra-assay within ± 10% of nominal value for low and high QC, n=20 samples

Sample Stability: Plasma is stable for up to 8 hours at ambient temperature (66 hours refrigerated) or can be frozen for long-term storage. Plasma can be shipped overnight on ice-packs or frozen. Do not keep plasma at room temperature for more than 8 hours.

Reportable Range:

7α,12α-DIHYDROXY-4-CHOLESTEN-3-ONE	10 ng/mL to 10,000 ng/mL

Reference Ranges:

Normal concentration in unaffected individuals age range 4.0-70.3 years old, n=14	< 10 ng/mL (< 0.024 nmol/mL)
Concentrations in CTX-affected untreated individuals age range 5.2-33.0 years old, n=14	3,561 ± 1,181 ng/mL [1,549-5,632] (8.548 ± 2.835 nmol/mL [3.719-13.519]) **mean ± SD [range]**

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PLASMA/SERUM BILE ACID PATHWAY INTERMEDIATE, 7α-DIHYDROXY-4-CHOLESTEN-3-ONE, FREE

Plasma (preferred)/serum	3 mL lavender or green top (plasma) 3 mL red top (serum) (minimum volume 0.5 mL)

Method: Addition of 7α-hydroxy-4-cholesten-3-one-d_7 method internal standard in methanol to sample and extracted as previously described. Isotope-dilution quantification is performed using positive-mode LC-ESI-MS/MS multiple reaction monitoring of 7αC4 (m/z 401.2>177.1 and 401.2>97.0) and internal standard (m/z 408.3>177.1) with a reversed-phase gradient utilizing a 150 x 4.6 mm Biphenyl (2.6 µm) column. Linear up to 1,000 ng/mL without dilution. No significant interference from common over the counter and prescription medications. Carry over response in blanks is < 10% of the LLOQ for the analyte and < 1% for the internal standard

References: DeBarber AE et al (2014) Clin Biochem. 47(9):860-3, Yuan L et al (2016) Bioanalysis. 8:2445-2455, Donato LJ et al (2018) Clin Biochem. 52:106-111.

Precision: In-house intra-assay CV < 8% for low QC n=20 samples and < 8% for high QC n=20 samples

Accuracy: In-house intra-assay within ± 10% of nominal value for low and high QC, n=20 samples

Sample Stability: Plasma is stable for up to 8 hours at ambient temperature (66 hours refrigerated) or can be frozen for long-term storage. Plasma can be shipped overnight on ice-packs or frozen. Do not keep plasma at room temperature for more than 8 hours.

Reportable Range:

7α-HYDROXY-4-CHOLESTEN-3-ONE	20 ng/mL to 10,000 ng/mL

Reference Ranges:

Normal concentration in unaffected individuals age range 4.0-70.3 years old, n=14	16.3 ±19.5 ng/mL[1] (0.041 ± 0.049 nmol/mL) **mean ± SD**
Concentrations in CTX-affected untreated individuals age range 5.2-33.0 years old, n=14	2,628 ± 1,244 ng/mL [1,215-4,863] (6.560 ± 3.104 nmol/mL [3.033-12.139]) **mean ± SD [range]**

1. 7α-hydroxy-4-cholesten-3-one normal reference range calculated including concentrations <LLOQ. Note that reported normal reference ranges are 22 ± 20 ng/mL (Mignarri A *et al* (2016) *J Inherit Metab Dis* **39(1):**75-83) and 15 ± 4 ng/mL (Matysik S *et al* (2011) *Chem Phys Lipids* **164(6):**530-4)

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PLASMA/SERUM BILE ALCOHOL

5β-CHOLESTANE-3α,7α,12α,25-TETROL GLUCURONIDE

Plasma (preferred)/serum	3 m lavender or green top (plasma) 3 mL red top (serum) (minimum volume 0.5 mL)

Method: Addition of 5β-cholestane-3α,7α,12α,25-tetrol-3-O-β-D-glucuronide-d_6 method internal standard in methanol to sample and extracted as previously described. Isotope-dilution quantification is performed using positive-mode LC-ESI-MS/MS multiple reaction monitoring of 5β-cholestane-3α,7α,12α,25-tetrol-3-O-β-D-glucuronide (m/z 611.4>75.0 and 611.4>85.0) and internal standard (m/z 617.4>75.0) with reversed-phase gradient utilizing a 150 x 4.6 mm Biphenyl (2.6 µm) column. Linear up to 5,000 ng/mL without dilution. No significant interference from common over the counter and prescription medications. Carry over response in blanks is < 2% of the LLOQ for the analyte and < 1% for the internal standard.

Reference: DeBarber AE et al (2018) J Lipid Res. 59 (11):2214-2222.

Precision: In-house intra-assay CV < 4% for low QC n=20 samples and < 5% for high QC n=20 samples

Accuracy: In-house intra-assay within ± 10% of nominal value for low and high QC, n=20 samples

Sample Stability: Plasma is stable for up to 8 hours at ambient temperature (66 hours refrigerated) or can be frozen for long-term storage. Plasma can be shipped overnight on ice-packs or frozen. Do not keep plasma at room temperature for more than 8 hours.

Reportable Range:

5β-CHOLESTANE-3α,7α,12α,25-TETROL GLUCURONIDE	200 ng/mL to 50,000 ng/mL

Reference Ranges:

Normal concentration in unaffected individuals age range 4.0-70.3 years old, n=14	< 200 ng/mL (< 0.326 nmol/mL)
Concentrations in CTX-affected untreated individuals age range 5.2-33.0 years old, n=14	10,536 ± 6,119 ng/mL [3,245-20,541] (17.193 ± 9.985 nmol/mL [5.296-33.520]) **mean ± SD [range]**

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URINARY BILE ALCOHOL

5β-CHOLESTANE-3α,7α,12α,23S,25-PENTOL, TOTAL

5-10 mL Random Urine, no preservatives (minimum volume 2 mL)

Method: Addition of 23S-pentol-d6 method internal standard followed by incubation of urine with β-glucuronidase enzyme. Isotope-dilution quantification performed using positive-mode LC-ESI-MS/MS multiple reaction monitoring of 23S-pentol (m/z 453.3>361.4) and internal standard (m/z 459.3>343.5) with a reversed-phase gradient utilizing a 4.6x50mm Biphenyl (2.6 µm) column. Linear up to 5,000 ng/mL without dilution. No significant interference from common over the counter and prescription medications. Carry over response in blanks is < 1% of the LLOQ for the analyte and < 0.5% for the internal standard.

References: Shimazu K et al (1986) J Biochem 99: 477, Batta AK et al (1985) J Lipid Res 26: 690.

Precision: In-house intra-assay CV < 4% for low QC n=20 samples and < 3% for high QC n=20 samples

Accuracy: In-house intra-assay within ± 10% of nominal value for low and high QC, n=20

Sample Stability: Urine is stable up to 5 days at ambient temperature or refrigerated. Samples can be frozen for long-term storage. Urine can be shipped over-night on ice-packs or frozen.

Reportable Range:

5β-CHOLESTANE-3α,7α,12α,23S,25-PENTOL	200 ng/mL to 250,000 ng/mL

Reference Ranges:

Normal concentration in unaffected individuals, n=20	< 200 ng/mL
Concentrations in CTX-affected untreated individuals age range 5.2-45.8 years old, n=14	58,941 ± 42,524 ng/mL [12,541-177,506] **mean ± SD [range]**

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