Brown Clinic Northridge
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BROWN CLINIC
LABORATORY PROCEDURE MANUAL

PROCEDURE: HDL Cholesterol

PURPOSE:
The AHDL method for the Dimension EXL200 Integrated chemistry system is an *in vitro* diagnostic test intended to quantitatively measure high density lipoprotein cholesterol (HDL-C) in **human serum** and **plasma**. HDL-C measurements are used as an aid in the diagnosis of lipid disorders(such as diabetes mellitus), various liver and renal diseases and in the assessment of risk for atherosclerosis and cardiovascular disease.

PRINCIPLE:
Plasma lipoproteins are spherical particles containing varying amounts of cholesterol, triglycerides, phospholipids and proteins. The phospholipid, free cholesterol and protein constitute the outer surface of the lipoprotein particle, while the inner core contains mostly esterified cholesterol and triglyceride. These particles serve to solubilize and transport cholesterol and triglyceride in the bloodstream.

The relative proportions of protein and lipid determine the density of these lipoproteins and provide a basis on which to begin their classification.[1] These classes are: chylomicron, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL).

The principle role of HDL in lipid metabolism is the uptake and transport of cholesterol from peripheral tissues to the liver through a process known as reverse cholesterol transport, a proposed cardiovascular protective mechanism. Low HDL-C levels are associated with an increased risk of coronary heart disease and coronary artery disease.[2-4]

Hence, the determination of serum HDL-C is a useful tool in identifying high-risk patients. The Adult Treatment Panel of the National Cholesterol Education Program (NCEP) recommends that all adults 20 years of age and over should have their total cholesterol and HDL cholesterol levels measured at least every 5 years to screen for coronary heart disease risk[5].

The reference method for measuring HDL-C utilizes ultracentrifugation and chemical precipitation to separate HDL from other lipoproteins, followed by cholesterol measurement using the Abell-Kendall assay. This method is too time consuming and labor intensive for use in routine analysis. Therefore, most laboratories utilize one of several methods for selective precipitation and removal of LDL and VLDL, followed by the enzymatic measurement of HDL-C in the supernatant fraction.[2-7]

The AHDL Cholesterol assay is a homogeneous method for directly measuring HDL-C levels without the need for off-line pretreatment or centrifugation steps.

The method is in a two reagent format and depends on the properties of a unique detergent, as illustrated. In the first reaction, chylomicrons, VLDL and LDL form water soluble complexes

with dextran sulfate in the presence of magnesium sulfate. These complexes are resistant to the polyethylene glycol (PEG)-modified cholesterol esterase and cholesterol oxidase that react with HDL cholesterol. In the presence of oxygen, the HDL cholesterol is oxidized to Δ 4-cholestenone and hydrogen peroxide. The generated hydrogen peroxide then reacts with 4-aminoantipyrine and sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HSDA) in the presence of peroxidase to form a colored dye that is measured using a bichromatic (600/700 nm) endpoint technique. The color intensity of the dye is directly proportional to the serum HDL-C concentration.

Accelerator Selective Detergent Methodology

HDL, LDL, VLDL, Chylomicrons —(Dextran Sulfate, Magnesium sulfate)→ Non-reactive LDL, VLDL, Chylomicrons + HDL Cholesterol esters

HDL cholesterol esters + H_2O —(PEG-Cholesterol esterase)→ HDL cholesterol + RCOOH

HDL Cholesterol +O_2 —(PEG-Cholesterol Oxidase)→ Δ^4 Cholestenone + H_2O_2

$2H_2O_2$ + 4-amino-antipyrine + HSDA+ H^+ + H_2O —(Peroxidase)→ Color development + 5 H_2O

INSTRUMENT, REAGENTS & SUPPLIES:

Wells[a]	Form	Ingredient	Concentration[b]	Source
1,2,3	Liquid	HEPES Buffer	10.07 mmol/L ph 7.4	
(Reagent 1)		2-(N-cyclohexylamino)-ethanesulfonic acid	96.95 mmol/L	
		Dextran Sulfate	1.5 g/L	
		Magnesium Nitrate Hexahdrate	≥11.7 mmol/L	
		N-(2-hydoxy-3-sulfopropyl)-3,5-dimethoxyaniline	0.96 mmol/L	
		Ascorbate Oxidase	≥50ukat/L	bacterial
		Peroxidase	≥16.7 ukat/L	horseradish
		Preservative		
4	Liquid	HEPES Buffer	10.07 mmol/L, ph 7.0	

(Reagent 2)		PEG-Cholesterol Esterase	≥3.33 ukat/L	bacterial
		PEG-Cholesterol Oxidase	≥127 ukat/L	bacterial
		Peroxidase	≥333 ukat/L	horseradish
		4-Amino-Antipyrine	2.46 mmol/L	
		Preservative		
5,6	Liquid	NaOH[c]	1.00 M	

a. Wells are numbered consecutively from the wide end of the cartridge.
b. Nominal value per well in a cartridge.
c. Sodium hydroxide is used as a probe cleaning solution and is not used in the reaction.

Risk and Safety:

H290, H314

P280, P304+P310, P301+P331,

P303+P361+P353+P310, P305+P310, P501

Danger!

May be Corrosive to Metals.
Causes severe skin burns and eye damage.

Wear protective gloves/protective clothing/eye protection/face protection. IF INHALED: remove victim to fresh air and keep at rest in a position comfortable for breathing. Immediately call a Poison Center or doctor/physician. IF SWALLOWED: immediately call a Poison Center or doctor/physician. Do not induce vomiting. IF ON SKIN OR HAIR: Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. Immediately call a Poison Center or doctor/physician. IF IN EYES: immediately calla poison center or doctor/physician. Dispose of contents and container in accordance with all local, regional and national regulations.

Contains: Sodium hydroxide

Safety data sheets (MSDS/SDS) available on www.siemens.com/healthcare

Precautions: Contains sodium azide (<0.1 %) as a preservative. Sodium azide can react with copper or lead pipes in drain lines to form explosive compounds. Dispose of properly in accordance with local regulations.

Used cuvettes contain human body fluids; handle with appropriate care to avoid skin contact or ingestion.

For *in vitro* diagnostic use

Reagent Preparation: All reagents are liquid and ready-to-use.

REAGENT STORAGE & STABILITY:

Store at 2 - 8 °C.

Expiration: Refer to carton for expiration date of individual unopened reagent cartridges. Sealed cartridge wells on the instrument are stable for 30 days.

Open Well Stability : 3 days for wells 1-3
10 days for well 4
15 days for wells 5-6

SPECIMEN REQUIREMENTS:

Blood should be collected after a 12-hour period of fasting by normal procedures.[8] Serum or plasma should be removed from cells within 2 hours of venipuncture. Serum, heparinized (lithium or sodium heparin) plasma are the recommended specimens.

Stability:

- Serum or plasma samples may be refrigerated at 2-8 °C for up to one week if not tested within 8 hours.
- For longer storage, samples may be frozen at -70 °C for up to three months. Samples may be frozen once. When using samples stored at 2-8 °C or -70°C, increases or decreases of up to 10% may be observed in HDL-C concentrations.

Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot-to-lot.

Specimen should be collected prior to administration of Metamizole due to potential for falsely decreased results.

Follow the instruction provided with your specimen collection device for use and processing.

CONTROLS:

At least once daily run solutions at two levels of a quality control material with known concentrations.

For further details, refer to your Dimension® system manual. The result obtained should fall within limits defined by the day-to-day variability of the system as measured in the user's laboratory. If the results fall outside the laboratory's acceptable limits, follow the procedure in the quality control policy.

PROCEDURE:

Materials Needed

AHDL Flex® reagent cartridge, Cat. No. DF48B
AHDL Calibrator, Cat. No. DC48B

Quality Control Materials

Test Steps

Sampling[d], reagent delivery, mixing, processing and printing of results are automatically performed by the Dimension® system. For details of this processing, refer to your Dimension® system manual.

d. The sample container must contain sufficient quantity to accommodate the sample volume plus dead volume. Precise container filling is not required.

Test Conditions

- Sample Size: 3 µL
- Reagent 1 Volume: 300 µL
- Reagent 2 Volume: 100 µL
- Test Temperature 37.0 ± 0.1 °C
- Reaction Time 8.6 minutes
- Wavelength 600 and 700 nm
- Type of measurement bichromatic, endpoint

Backup:

Refer to Brown Clinic Backup Policy

Calibration

The general calibration procedure is described in the Dimension® Operator's Guide. The following information should be considered when calibrating the HDL-C method:

- Measurement Range: 3 -150 mg/dL [0.08 - 3.89 mmol/L][e]
- Calibration Material: AHDL Calibrator, Cat. No. DC48B
- Calibration Scheme: 3 levels n =3
- Units: mg/dL [mmol/L]
  (mg/dLx 0.0259)= [mmol/L]
- Typical Calibration Levels: 0, 40, 165 mg/dL
  [0.00, 1.04, 4.27 mmol/L]
- Calibration Frequency: Every new reagent cartridge lot

  Every 90 for any one lot

  For each new lot of Flex reagent cartridges

  After major maintenance or service, if indicated by quality control results

  As indicated in laboratory quality control procedures

  When required by government regulations
- Assigned Coefficients: C_0 0.000
  C_1 1.700

e. Systeme International d'Unites [SI Units] are in brackets

INTERPRETATION:

The instrument automatically calculates and prints the concentration of HDL-C in mg/dL [mmol/L] using the calculation scheme illustrated in the Dimension® system manual.

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation, and other findings.

REFERENCE RANGE:

The National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III)[9] provides the following classifications of HDL-C concentrations:

HDL < 40 mg/dL [1.04 mmol/L] Low HDL Cholesterol
HDL ≥ 60 mg/dL [1.55 mmol/L] High HDL Cholesterol

REPORTING:
report format: mg/dl

LIMITATIONS:

Results:	>150 mg/dL [3.89 mmol/L]
Manual dilution:	Make appropriate dilution with purified water to obtain results within assay range. Enter dilution factor. Reassay. Resulting readout is corrected for dilution. HDL-C less than 10 mg/dL [0.26 mmol/L] should be reported as "less than 10 mg/dL [0.26 mmol/L]" instead of the numerical value.
Autodilution (AD):	Refer to your Dimension® system manual. The recommended autodilute sample volume is 2 µL.

The instrument reporting system contains error messages to warn the operator of specific malfunctions. Any report slip containing such error messages should be held for follow-up. Refer to your Dimension® system manual.

Analytical Specificity

For Known Interfering Substances section refer to package insert.

For Known Non-Interfering Substance refer to package insert.

For Additional Technical Information refer to package insert

Reference: AHDL Flex® reagent cartridge insert sheet PN 717048.101 Issue Date 2016-10-20

Origination Date: 9-10-07
Date of Implementation: 11-10-10
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Written By: ____Lori Murray MT(ASCP)_____ Date: _8-8-12

Approved By: __Aaron Shives, MD___ Date: __10/23/2017___
Laboratory Director

REVIEW - REVISION SUMMARY DOCUMENTATION

Date	By	Revision Summary
07/10	Lori Murray	New format
8-8-12	Lori Murray	Changed instrumentation to EXL200
5/6/15	Sam Legg	Updated information to package insert dated 2013-7-25
6/10/15	Heather Hall	Added to Risk and Safety section
8/26/15	Amy Harms	Updated Risk and Safety section package insert dated 2/25/15
10/17/17	Heather Hall	updated information to package insert dated 10-20-2016 Modified Backup process