HDL Cholesterol (HDL) – Serum, Plasma
Beckman UniCel DxC Systems

Technical Procedure 3130

Principle

Intended Use

HDL Cholesterol reagent, when used in conjunction with UniCel DxC SYNCHRON 800 System(s) and UniCel DxC SYNCHRON® Systems HDL Calibrator, is intended for quantitative determination of HDL Cholesterol in the high density lipoprotein fraction of human serum or plasma.

Clinical Significance

Many epidemiological investigations have demonstrated the strong and independent inverse association between HDL-Cholesterol and the risk of coronary artery disease. (1,2) It has be proposed that HDL particles, through uptake and transport of cholesterol from peripheral tissue to the liver (reverse cholesterol transport), protects against the development of atheromatous plaques. (3)

The National Cholesterol Education Program (NECP) guidelines classify HDL cholesterol levels as follows (4,5):

< 40 mg/dL as indicative of a major risk factor for Coronary Heart Disease

≥ 60 mg/dL as a negative risk factor for Coronary Heart Disease.

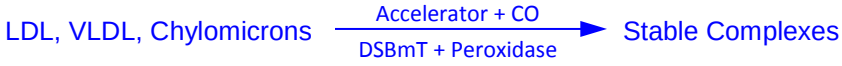
Methodology

This HDL cholesterol test is a two reagent homogenous system for the selective measurement of serum or plasma HDL cholesterol in the presence of other lipoprotein particles. The assay is comprised of two distinct phases. In phase one, free cholesterol in non-HDL lipoproteins is solubilized and consumed by cholesterol oxidase, peroxidase, and DSBmT to generate a colorless end product. In phase two, a unique detergent selectively solubilizes HDL. The HDL cholesterol is released for reaction with cholesterol esterase and cholesterol oxidase, in the presence of chromogens, to produce a colored product.

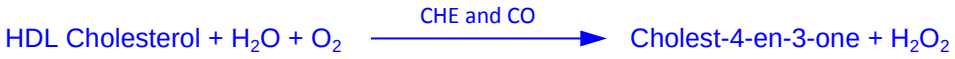
The HDL reagent measures the HDL cholesterol concentration by a timed-endpoint method. (6) The system automatically proportions the appropriate HDL cholesterol sample and reagent volumes into a cuvette. The ratio used is one part sample to 93 parts reagent. The system monitors the change in absorbance at 560 nanometers. This change in absorbance is directly proportional to the concentration of cholesterol in the sample and is used by the system to calculate the HDL cholesterol concentration.

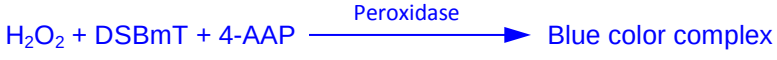
Chemical Reaction Scheme

Reaction Phase 1



Reaction Phase 2





DSBmT = N,N-bis(4-sulphobutyl)-m-toluidine-disodium
4-AA = 4-aminoantipyrine

Specimen

Acceptable Sample Containers

13 x 75 PST, SST and Red Top BD tubes
PST, SST and Red Top BD microtainers
Optimum volume: 0.5 mL, Minimum volume: 0.3 mL

Type of Specimen

Biological fluid samples should be collected in the same manner routinely used for any laboratory test.(7) Freshly drawn serum, or plasma are the preferred specimens. Whole blood and urine are not recommended for use as a sample.
Acceptable anticoagulants are listed in *Procedural Notes* section of this chemistry information sheet.

Patient Preparation

None required.

Note: If lipid panel including Triglyceride and Direct LDL is ordered, the patient must fast for at least 12 hours. The fasting status of the patient must be noted on the order request when the blood is collected.

Specimen Storage and Stability

Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum or plasma should be physically separated from contact with cells within two hours from the time of collection.(8)

Separated serum or plasma should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at 2°C to 8°C. If assays are not completed within 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once.(9) Analyte deterioration may occur in samples that are repeatedly frozen and thawed.

Thawed samples should be mixed gently and re-centrifuged prior to analysis.

Sample Volume

The optimum volume, when using a 0.5 mL sample cup, is 0.3 mL of sample. For optimum primary sample tube volumes and minimum volumes, refer to the *Primary Tube Sample Template*.

If a lipid panel is ordered, the optimum volume is 0.5 mL of sample.

Unacceptable Specimens

Refer to the *Procedural Notes* section of this chemistry information sheet for information on unacceptable specimens.

Reagents

Contents

Each kit contains the following items:
Two HDL Reagent Cartridges (2 x 200 tests) **Kit Reorder # A15625**

Volumes per Test

Sample Volume	3 µL
Total Reagent Volume	280 µL

Cartridge Volumes	
A	210 µL
B	70 µL
C	---

Reactive Ingredients

Reagent Constituents	
Cholesterol Esterase (Pseudomonas)	375 U/L
Cholesterol Oxidase (E. coli)	750 U/L
Peroxidase (horseradish)	975 U/L
Ascorbate oxidase (Cucurbita sp.)	2250 U/L
DSBmT	0.75 mmol/L
4-aminoantipyrine	0.25 mmol/L
Detergent	0.375%
Preservative	0.05%

Also non-reactive chemicals necessary for optimal system performance.

Materials Needed But Not Supplied With Reagent Kit

UniCel DxC SYNCHRON Systems HDL Calibrator
Deionized water
At least two levels of control material
Saline

Reagent Preparation

No preparation is required.
Date and initial reagent container(s) and document in reagent log before loading each new cartridge.

Acceptable Reagent Performance

The acceptability of this reagent is determined by successful calibration and by ensuring that quality control results are within acceptance criteria, as defined in the Clinical Chemistry Quality Control Procedure #3000.T.

Reagent Storage and Stability

HDL Cholesterol reagent when stored unopened at 2°C to 8°C will attain the shelf-life indicated on the cartridge label. Once prepared, the reagent is stable for 60 days at 2°C to 8°C unless the expiration date is exceeded.

DO NOT FREEZE.

Equipment

This test is performed on the Beckman UniCel DxC 800 systems; Beckman-Coulter, Brea, California. For technical assistance, call the Beckman-Coulter hotline: 1-800-854-3633.

Refer to the UniCel DxC 800 systems *Reference Manual* for detailed instructions.

Calibration

Calibrator Required

SYNCHRON® Systems HDL Calibrator **Reference #B23634**
Use deionized water (Level 1 calibrator)

Calibrator Preparation

Use deionized water as the Level 1 calibrator.

1. Remove the metal cap around the HDL calibrator bottle and gently tap the bottle to remove powder at the top of the stopper. Open the HDL calibrator bottle carefully, avoiding loss of lyophilizate.
2. Add exactly 1.00 mL of deionized water to the bottle of calibrator. Replace the stopper and let stand for 5 minutes at room temperature.
3. Gently invert until the contents are dissolved avoiding the formation of foam. DO NOT SHAKE or VORTEX.

Calibrator Storage and Stability

If unopened, the SYNCHRON Systems Lipid Calibrator may be stored at 2°C to 8°C until the expiration date printed on the calibrator bottle. Reconstituted calibrators that are resealed are stable for 14 days at 2°C to 8°C or for 30 days at -15°C to -20°C unless the expiration date is exceeded or when calibration and quality control recoveries have shifted. (9) Frozen calibrators should be thawed only once.

CAUTION

Because this product is of human origin, it should be handled as though capable of transmitting infectious diseases. Each serum or plasma donor unit used in the preparation of this material was tested by United States Food and Drug Administration (FDA) approved methods and found to be negative for antibodies to HIV and HCV and nonreactive for HbsAg. Because no test method can offer complete assurance that HIV, hepatitis B virus, and hepatitis C virus or other infectious agents are absent, this material should be handled as though capable of transmitting infectious diseases. This product may also contain other human source material for which there is no approved test. The FDA recommends such samples to be handled as specified in Centers for Disease Control's Biosafety Level 2 guidelines.(10)

Calibration Information

The system must have a valid calibration in memory before controls or patient samples can be run.

Under typical operating conditions the HDLD assay must be calibrated every 28 days or with each new cartridge of reagent, and also with certain parts replacement or maintenance procedures, as defined in the UniCel DxC800 System *Instructions for Use* (IFU) manual. This assay has within-lot calibration available. Refer to the UniCel DxC 800 System *Instructions for Use* (IFU) manual for information on this feature.

For detailed calibration instructions, refer to the UniCel DxC800 System *Instructions for Use* (IFU) manual.

The system will automatically perform checks on the calibration and produce data at the end of calibration. In the event of a failed calibration, the data will be printed with error codes and the system will alert the operator of the failure. For information on error codes, refer to the UniCel DxC800 System *Instructions for Use* (IFU) Manual.

Traceability

HDL Cholesterol measurand (analyte) in this calibrator is traceable to NIST* SRM 911b. The traceability process is based on prEN ISO 17511.

The assigned values were established using representative samples from this lot of calibrator and are specific to the assay methodologies of the SYNCHRON LX and the UniCel DxC reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

*NIST – National Institute of Standards and Technology.

Quality Control

At least two levels of control material should be analyzed each shift. In addition, these controls should be run with each new calibration, with each new reagent cartridge, and after specific maintenance or troubleshooting procedures as detailed in the UniCel DxC 800 System *Instructions For Use* manual. More frequent use of controls or the use of additional controls is left to the discretion of the user based on workload and workflow or laboratory accreditation requirements and applicable laws.

The following controls should be used in accordance with the package instructions for use inserts. Quality control results should be evaluated and handled with respect to the Clinical Chemistry Quality Control Procedure #3000.T.Controls are compiled statistically in the LIS and reagent lot changes are documented on DxC Reagent Log sheets.

Quality Control Material

Control	Storage
MAS ChemTrak 1	2°C to 8°C*
MAS ChemTrak 3	2°C to 8°C*

*Controls are received frozen and stored at –15°C to –25°C.
Bottles of controls in use are thawed and stored at 2°C to 8°C and are good for 14 days.

Testing Procedure

1. If necessary, load the reagent onto the system.
2. After reagent load is completed, calibration is required.
3. Program samples and controls for analysis.
4. After loading samples and controls onto the system, follow the protocols for system operation.

For detailed testing procedures, refer to the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

Calculations

UniCel DxC Systems perform all calculations internally to produce the final reported result. The system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

If the dilution was programmed in Remisol, the final calculated result from a dilution will not be calculated by the UniCel DxC system but by Remisol.

Lipid Panel Calculations are performed in the LIS

Calculation of LDL-c: This is only calculated on fasting specimens with triglyceride levels less than or equal to 400 mg/dL and non-fasting specimens with triglyceride levels less than or equal to 150 mg/dL. Friedewald, Levy, and Fredrickson in 1972 established and validated a formula to calculate LDL cholesterol (LDLcalc). The formula is based on the assumption the VLDL cholesterol is present in a concentration equal to one-fifth of the triglyceride concentration. This assumption is valid for triglyceride concentrations of less than or equal to 400 mg/dL. Above this level, inconsistencies in the VLDL triglyceride/cholesterol ratio occur and the formula cannot be used. It is also not valid for Type III and certain other lipid disorders. To calculate LDL-c (for triglycerides ≤ 400 mg/dL[fasting] and for triglycerides ≤150 mg/dL[non-fasting]):

$$LDL(calc) = Cholesterol_{TOTAL} – [HDL + Triglyceride/5]$$

Total cholesterol/HDL ratio:

$$\text{Total cholesterol/HDL ratio} = \frac{\text{Total Cholesterol (mg/dL)}}{\text{HDL (mg/dL)}}$$

Calculation of VLDL-C (not part of current lipid panel): This can be calculated when the triglyeride level is ≤ 400 mg/dL and when a direct LDL result is obtained on a sample with the triglyceride result between 401 and 1293 mg/dL:

VLDL(calc) = Total Cholesterol – HDL – Direct LDL

LDL/HDL ratio: this can be calculated as Friedewald LDL - HDL when the triglyceride level is ≤ 400 mg/dL. When a Direct LDL assay is performed, the LDL-D value is used in the ratio instead of the calculated LDL.

Non-HDL Cholesterol: calculated result (to conform with the latest NCEP ATPIII guidelines)

Non-HDL Cholesterol = [Total Cholesterol – HDL Cholesterol]

The clinician will be able to order a Lipid Panel that reflexes to a direct LDL if the LDL calculation is not performed. The direct LDL will only be performed on samples if the fasting tryiglyceride is > 400 mg/dL and ≤ 1293 mg/dL, or for non-fasting samples if the triglyceride is > 150 mg/dL and ≤ 1293 mg/dL.

Reporting Results

Equivalency between the SYNCHRON LX and UniCel DxC 800 Systems has been established. Chemistry results between these systems are in agreement and data from representative systems may be shown.

Reference Intervals

Reference intervals established at UCDMC: (also to conform to the latest NCEP ATPIII guidelines)

HDL (males)	≥ 35 mg/dL
HDL (females)	≥ 35 mg/dL
LDL-C	< 130 mg/dL
$Cholesterol_{TOTAL}$/HDL	Desirable ratio < 4.0
VLDL reference interval	7 – 32 mg/dL*
Non-HDL Cholesterol (males)	≤ 160 mg/dL
Non-HDL Cholesterol (females)	≤ 150 mg/dL

* Determined by the fasting triglyceride reference interval (35 to 160 mg/dL) divided by 5.

Procedural Notes

Anticoagulant Test Results

The following anticoagulants were assessed by Deming regression analysis with a minimum of 50 paired serum and plasma samples. Values of serum (X) ranging from 17 mg/dL to 127 mg/dL were compared with the values from plasma (Y) yielding the following results:

Acceptable Anticoagulants

Anticoagulant	Level of Anticoagulant tested	Deming Regression Analysis
Lithium Heparin	17 Units/mL	Y = 0.995X + 0.3; r = 0.998
Sodium Heparin	17 Units/mL	Y = 0.994X - 0.3; r = 0.998

Limitations

Samples or control materials which contain acetic acid, detergents, or surfactants may inhibit the enzymes in the reagent and should not be used.

Grossly lipemic samples should be ultracentrifuged and re-analyzed with triglycerides. Result will be acceptable if the triglyceride result is less than 1700 mg/dL. If triglyceride is > 1700 mg/dL from the ultracentrifuged sample, dilute with saline and re-analyze.

Extremely lipemic samples with triglycerides greater than 1700 mg/dL may give falsely low results. Dilute samples with saline.

Interferences

The following substances were tested for interference with this methodology:

Interferences

Substance	Source	Level Tested	Observed Effect
Hemoglobin	RBC Hemolysate	500 mg/dL	NSI[a]
Bilirubin	Mixed Isomers	40 mg/dL	NSI
Triglyceride	Human	900 mg/dL	NSI
Lipemia	Intralipid[b]	1500 mg/dL	NSI
Ascorbic Acid	N/A[c]	20 mg/dL	NSI
Immunoglobulin IgG	Human	5000 mg/dL	NSI

[a] NSI = No Significant Interference is recovery within 10% of the initial result.
[b] Company and product names are the property of their respective owners.
[c] NA = Not applicable

Inaccurate results (usually negative interference) may be produced in patients with elevated serum immunoglobulin levels.(15) In very rare cases gammopathy, especially monoclonal IgM (Waldeström's macroglobumlinemia) may cause unreliable results.

Falsely low results may be obtained in patients with Type III hyperlipidemia.(16)

Refer to References (17,18,19) for other interferences caused by drugs, disease and preanalytical variables.

Performance Characteristics

Analytical Measurement Range

The UniCel DxC SYNCHRON System(s) method for the determination of HDL provides the following analytical ranges, which have been verified using CLSI EPO6-A (20):

Analytical measurement Range (AMR)

Sample Type	Conventional Units
Serum, Plasma or Urine	5-135 mg/dL

Clinical Reportable Range:

Clinical Reportable Range (CRR)

Sample Type	Conventional Units
Serum, Plasma or Urine	5- diluted result mg/dL

Samples with concentrations below the AMR and CRR (5 mg/dL) will be reported as "**< 5 mg/dL**".

Samples with concentrations greater than the AMR should be diluted with saline and reanalyzed.

If the dilution was programmed in Remisol, the final calculated result from a dilution will not be calculated by the UniCel DxC system but by Remisol.

Sensitivity

Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95%confidence. Sensitivity for HDLD determination is 5 mg/dL.

Equivalency

Equivalency was assessed by Deming regression analysis of patient samples to accepted clinical methods.

As determined by Beckman

Serum or plasma (in the range of 8.0 to 123.7 mg/dL):

Y (UniCel DxC 800 Systems)	= 1.015X – 0.9
N	= 131
MEAN Y (UniCel DxC 800 System)	= 56.0
MEAN X (AU680 OSR6x95))	= 56.1
CORRELATION COEFFICIENT (r)	= 0.999

Refer to References (23) for guidelines on performing equivalency testing.

Precision

A properly operating SYNCHRON® System(s) should exhibit precision values less than or equal to the following:

As determined by Beckman

Precision Values

Type of Precision	Sample Type	1 SD	Changeover Value[a]	%CV
		mg/dL	mg/dL	
Within-run	Serum/Plasma	1.3	42	3.0
Total	Serum/Plasma	1.7	42	4.0

[a]When the mean of the test precision data is less than or equal to the changeover value, compare the test SD to the SD guideline given above to determine the acceptability of the precision testing. When the mean of the test precision data is greater than the changeover value, compare the test % CV to the guideline given above to determine acceptability. Changeover value = (SD guideline/CV guideline) x 100.

Comparative performance

Comparative performance data for the system evaluated using the NCCLS Approved Guideline EP5-A2 appears in the following table.(24) Each laboratory should characterize their own instrument performance for comparison purposes.

NCCLS EP5-A Precision Estimate Method

Type of Imprecision	Sample Type	n[a]	Mean (mg/dL)	EP5-T2 Calculated Point Estimates	
				SD	%CV
Within-Run	Serum Control 1	80	33.3	0.3	0.9
	Serum Control 2	80	56.1	0.6	1.1
	Serum Control 3	80	121.0	1.2	1.0
Total	Serum Control 1	80	33.3	0.6	1.8
	Serum Control 2	80	56.1	1.0	1.8
	Serum Control 3	80	121.0	1.6	1.3

[a]The point estimate is based on the pooled data from one system, run for twenty days, two runs per day, two observations per run on aninstrument operated and maintained according to the manufacturer's instructions

NOTICE

These degrees of precision and equivalency were obtained in typical testing procedures on a SYNCHRON LX® System and are not intended to represent the performance specifications for this reagent.

Comparison of HDLD to HDL

In 2015, Beckman Coulter updated the assay from HDLD to HDL. Precision and accuracy studies were performed. Overall, HDLD and HDL performance were similar. Results of these comparisons are summarized in *Appendices A and B* at the end of this document.

Additional Information

For more detailed information on UniCel DxC Systems, refer to the *Instructions for Use* and *Reference* manual.

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Prepared By	Date Adopted	Supersedes Procedure #
Michael Inn	October, 2001	Renumbered from 3300.T

Revision Date	Type of Revision	Revised by	Review/Annual Review Date	Reviewed By
			11/27/2000	G.Kost
			12/28/2001	G.Kost
			10/16/2002	G.Kost
			10/10/2003	S.Devaraj
10/2004		M. Inn		
			10/25/2004	S.Devaraj
			11/28/2005	G. Kost
			09/26/2006	G. Kost
			11/05/2007	G. Kost
			06/16/2008	G. Kost
			09/15/2009	G. Kost
			10/12/2010	G. Kost
			11/16/2011	G. Kost
09/14/2013	General update	M. Inn	09/17/2013	G. Kost
07/30/2015	calibrator and method update	kdagang	08/24/2015	J. Gregg