

For *In Vitro* Diagnostic Use

ANNUAL REVIEW

Reviewed by:	Date	Reviewed by:	Date

PRINCIPLE

INTENDED USE

HDLD reagent, when used in conjunction with SYNCHRON LX[®] System(s), UniCel[®] DxC 600/800 System(s) and SYNCHRON[®] Systems Lipid Calibrator, is intended for quantitative determination of hdl cholesterol in the high density lipoprotein fraction of human serum or plasma.

CLINICAL SIGNIFICANCE

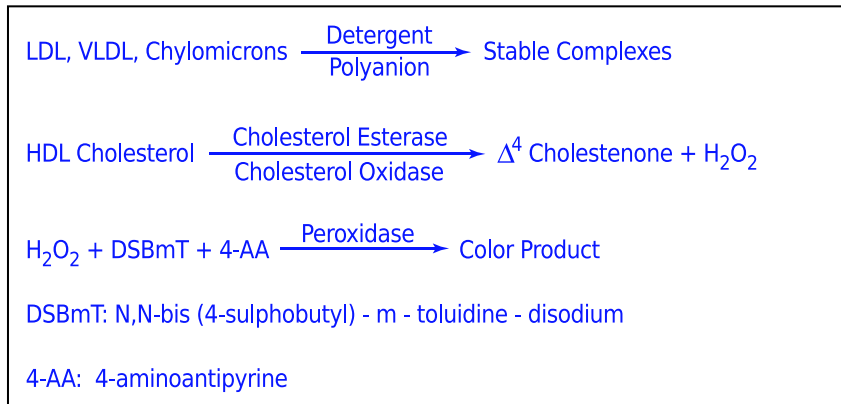
HDL cholesterol is inversely related to the risk of developing coronary artery disease¹. A low HDL/LDL cholesterol ratio is directly related to the risk of developing coronary artery disease. A high HDL cholesterol is associated with the "longevity" syndrome.

METHODOLOGY

This direct HDL Cholesterol method is a homogeneous assay without the need for any offline pretreatment or centrifugation steps. The method depends on a unique detergent which solubilizes only the HDL lipoprotein particles and releases HDL cholesterol to react with cholesterol esterase and cholesterol oxidase in the presence of chromogens, to produce a color product. The same detergent also inhibits the reaction of the cholesterol enzymes with LDL, VLDL, and chylomicrons lipoproteins by adsorbing to their surfaces. A polyanion contained in the reagent enhances the selectivity for HDL cholesterol assay by complexing LDL, VLDL, and chylomicrons lipoproteins.

HDLD reagent is used to measure the cholesterol concentration by a timed-endpoint method.^{2,3} The SYNCHRON[®] System(s) 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 and express the HDL-cholesterol concentration.

CHEMICAL REACTION SCHEME



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SPECIMEN

TYPE OF SPECIMEN

Biological fluid samples should be collected in the same manner routinely used for any laboratory test.⁴ Freshly drawn serum or plasma are the preferred specimens. Acceptable anticoagulants are listed in the PROCEDURAL NOTES section of this chemistry information sheet. Whole blood or urine are not recommended for use as a sample.

SPECIMEN STORAGE AND STABILITY

1. Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum or plasma be physically separated from contact with cells within two hours from the time of collection.⁵
2. 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. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.⁵

Additional specimen storage and stability conditions as designated by this laboratory:

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 for your system.

CRITERIA FOR UNACCEPTABLE SPECIMENS

Refer to the PROCEDURAL NOTES section of this chemistry information sheet for information on unacceptable specimens.

Criteria for sample rejection as designated by this laboratory:

PATIENT PREPARATION

Special instructions for patient preparation as designated by this laboratory:

SPECIMEN HANDLING

Special instructions for specimen handling as designated by this laboratory:

REAGENTS

CONTENTS

Each kit contains the following items:

Two HDLD Reagent Cartridges (2 x 200 tests)

VOLUMES PER TEST

Sample Volume	3 μ L
Total Reagent Volume	280 μ L
Cartridge Volumes	
A	210 μ L
B	70 μ L
C	--

REACTIVE INGREDIENTS

REAGENT CONSTITUENTS

Cholesterol esterase (bacterial)	1250 IU/L
Cholesterol oxidase (bacterial)	1600 IU/L
Peroxidase (horseradish)	5000 IU/L
Polyanion	0.1%
Detergent	1.0%

Also non-reactive chemicals necessary for optimal system performance.

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

SYNCHRON® Systems Lipid Calibrator
At least two levels of control material
Saline

REAGENT PREPARATION

No preparation is required.

ACCEPTABLE REAGENT PERFORMANCE

The acceptability of a reagent is determined by successful calibration and by ensuring that quality control results are within your facility's acceptance criteria.

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 opened, the reagent is stable for 60 days at +2°C to +8°C unless the expiration date is exceeded. **DO NOT FREEZE.**

Reagent storage location:

CALIBRATION

CALIBRATOR REQUIRED

SYNCHRON® Systems Lipid Calibrator

CALIBRATOR PREPARATION

No preparation is required.

CALIBRATOR STORAGE AND STABILITY

If unopened, the SYNCHRON® Systems Lipid Calibrator may be stored at -15°C to -20°C until the expiration date printed on the calibrator bottle. Opened calibrators that are resealed and stored at +2°C to +8°C are stable for 60 days unless the expiration date is exceeded.

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.⁶

Calibrator storage location:

CALIBRATION INFORMATION

1. The system must have valid calibration factors in memory before controls or patient samples can be run.
2. Under typical operating conditions the HDLD assay must be calibrated every 30 days or with each new cartridge of reagent and also with certain parts replacements or maintenance procedures, as defined in the SYNCHRON LX *Maintenance Manual and Instrument Log*, or the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual. This assay has within-lot calibration available. Refer to the SYNCHRON LX *Operations Manual*, or the UniCel DxC 600/800 Systems *Instructions For Use* (IFU) manual for information on this feature.
3. For detailed calibration instructions, refer to the SYNCHRON LX *Operations Manual*, or the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.
4. 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 SYNCHRON LX *Diagnostics and Troubleshooting Manual*, or the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

TRACEABILITY

For Traceability information refer to the Calibrator instructions for use.

QUALITY CONTROL

At least two levels of control material should be analyzed daily. 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 appropriate system manual. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws.

The following controls should be prepared and used in accordance with the package inserts. Discrepant quality control results should be evaluated by your facility.

Table 1.0 Quality Control Material

CONTROL NAME	SAMPLE TYPE	STORAGE

TESTING PROCEDURE(S)

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

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

CALCULATIONS

The SYNCHRON® System(s) performs 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.

REPORTING RESULTS

Equivalency between the SYNCHRON LX and UniCel DxC 600/800 Systems has been established. Chemistry results between these systems are in agreement and data from representative systems may be shown.

REFERENCE INTERVALS

Each laboratory should establish its own reference intervals based upon its patient population. The reference interval listed below was taken from a study performed on SYNCHRON Systems.

Table 2.0 Reference Intervals^{7,8}

INTERVALS	CARDIOVASCULAR RISK	CONVENTIONAL UNITS	S.I. UNITS
SYNCHRON	Low	≥ 60 mg/dL	≥ 1.55 mmol/L
	High	< 40 mg/dL	< 1.03 mmol/L

INTERVALS	CARDIOVASCULAR RISK	CONVENTIONAL UNITS	S.I. UNITS
Laboratory			

Refer to References (9,10,11) for guidelines on establishing laboratory-specific reference intervals.

Additional reporting information as designated by this laboratory:

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 26 mg/dL to 127 mg/dL were compared with the values for plasma (Y) yield the following results.

Table 3.0 Anticoagulant Test Results

ANTICOAGULANT	LEVEL OF ANTICOAGULANT TESTED	DEMING REGRESSION ANALYSIS
Lithium Heparin	14 Units/mL	$Y = 1.012X + 0.7; r = 0.998$
Sodium Heparin	14 Units/mL	$Y = 1.030X - 0.3; r = 0.997$

LIMITATIONS

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

INTERFERENCES

1. The following substances were tested for interference with this methodology:

Table 4.0 Interferences

SUBSTANCE	SOURCE	LEVEL TESTED	OBSERVED EFFECT
Hemoglobin	RBC hemolysate	500 mg/dL	NSI ^a
Bilirubin	Porcine	30 mg/dL	NSI
Lipemia	Human	Triglyceride \leq 1700	NSI
Ascorbic Acid	NA ^b	50 mg/dL	NSI
Immunoglobulin IgG	Human	3000 mg/dL	NSI

a NSI = No Significant Interference (within ± 6.0 mg/dL or 6%).

b NA = Not applicable.

2. Extremely lipemic samples with triglycerides greater than 1700 mg/dL may give falsely low results.
3. Inaccurate results (usually negative interference) may be produced in patients with elevated serum immunoglobulin levels.¹²
4. Falsely low results may be obtained in patients with Type III hyperlipidemia.¹³
5. Refer to References (14,15,16) for other interferences caused by drugs, disease and preanalytical variables.

PERFORMANCE CHARACTERISTICS

ANALYTIC RANGE

The SYNCHRON[®] System(s) method for the determination of this analyte provides the following analytical ranges:

Table 5.0 Analytical Range

SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS
Serum or Plasma	5 – 135 mg/dL	0.13 – 3.50 mmol/L

Samples with concentrations exceeding the high end of the analytical range should be diluted with saline and reanalyzed.

REPORTABLE RANGE (AS DETERMINED ON SITE):

Table 6.0 Reportable Range

SAMPLE TYPE	CONVENTIONAL UNITS	S.I. UNITS

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 (0.13 mmol/L).

EQUIVALENCY

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

Serum or plasma (in the range of 5 to 83 mg/dL):

Y (SYNCHRON LX Systems)	= 0.973X + 0.5
N	= 66
MEAN (SYNCHRON LX HDLD)	= 40.3
MEAN (SYNCHRON LX HDLC)	= 41.0
CORRELATION COEFFICIENT (r)	= 0.972

Serum or plasma (in the range of 7 to 130 mg/dL):

Y (SYNCHRON LX Systems)	= 0.893X + 6.7
N	= 80
MEAN (SYNCHRON LX HDLD)	= 52.4
MEAN (SYNCHRON LX HDLD UDR Method)	= 51.2
CORRELATION COEFFICIENT (r)	= 0.999

Refer to References (17) for guidelines on performing equivalency testing.

PRECISION

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

Table 7.0 Precision Values

TYPE OF PRECISION	SAMPLE TYPE	1 SD		CHANGEOVER VALUE ^a		% CV
		mg/dL	mmol/L	mg/dL	mmol/L	
Within-run	Serum/Plasma	3.0	0.07	100.0	2.3	3.0
Total	Serum/Plasma	4.5	0.11	100.0	2.3	4.5

^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 data for a SYNCHRON LX[®] System evaluated using the NCCLS Proposed Guideline EP5-T2 appears in the table below.¹⁸ Each laboratory should characterize their own instrument performance for comparison purposes.

Table 8.0 NCCLS EP5-T2 Precision Estimate Method

TYPE OF IMPRECISION	SAMPLE TYPE		No. Systems	No. Data Points ^a	Test Mean Value (mg/dL)	EP5-T2 Calculated Point Estimates	
						SD	%CV
Within-run	Serum	Control 1	1	80	24.5	0.5	2.0
	Serum	Control 2	1	80	56.0	1.0	1.8
	Serum	Control 3	1	80	122.8	2.1	1.7
Total	Serum	Control 1	1	80	24.5	0.8	3.4
	Serum	Control 2	1	80	56.0	1.2	2.1
	Serum	Control 3	1	80	122.8	2.8	2.2

^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 an instrument 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.

ADDITIONAL INFORMATION

For more detailed information on SYNCHRON LX Systems or UniCel DxC Systems, refer to the appropriate system manual.

SHIPPING DAMAGE

If damaged product is received, notify your Beckman Coulter Clinical Support Center.

REFERENCES

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18. National Committee for Clinical Laboratory Standards, *Precision Performance of Clinical Chemistry Devices*, Tentative Guideline, 2nd Edition, NCCLS publication EP5-T2, Villanova, PA (1992).



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