DXC 800 (HDL) HDL CHOLESTEROL

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PURPOSE

To provide instructions for the quantitative determination of HDL cholesterol on the DXC 800.

PRINCIPLE

HDL reagent, when used in conjunction with 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.

BACKGROUND

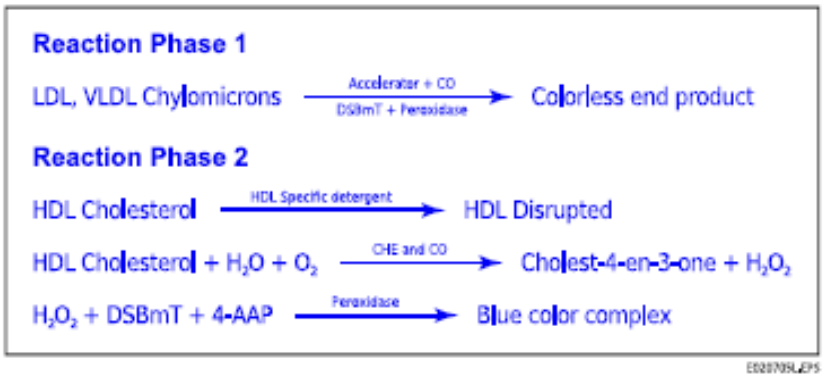
Clinical Significance

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 been proposed that HDL particles, through the uptake and transport of Cholesterol from peripheral tissue to the liver (reverse Cholesterol transport), protects against the development of atheromatous plaques.3
The guidelines issued by The National Cholesterol Education Program Adult Treatment Panel 3 (NCEP ATP 3),4 recommends lipoprotein analysis (total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides) as the preferred initial test, rather than screening for total cholesterol and HDL alone. In 2001, the NCEP increased the high-risk medical decision point to <40 mg/dL.5
The guidelines classify HDL- C levels as follows:
1. < 40 mg/dL as indicative of a major risk factor for Coronary Heart Disease.
2. ≥ 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 colour 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 and express the HDL cholesterol concentration.



RELATED DOCUMENTS

R-PO-CH-0810	Quality Control Program General Laboratory
R-PO-CH-0809	Quality Control Westgard Rules Statistics
R-PR-AD-0540	Specimen Rejection/Cancellation Protocol
J-F-CH-0820	DXC 800 Controls
J-F-CH-0826	DXC 800 Calibrators
J-F-CH-1940	DXC Analytical Measurement Range

SPECIMEN

Type of Specimen

Biological fluid samples should be collected in the same manner routinely used for any laboratory test. 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.

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.

3. HDL fractions are stable for 48 hours at +2°C to +8°C.

Sample Type	Volume	Sample Stability
Plasma	0.5mL	• Separate serum from cells within 2 hours. • Room Temp 8 hours • Refrigerated 48 hours • Frozen 3 months.

Criteria for Unacceptable Specimens

See Specimen Rejection/Cancellation Protocol

Sample Volume

A filled 0.5 mL sample cup is the optimum volume. For optimum primary sample tube volumes in primary tube samples and minimum volumes, refer to the Primary Tube Sample Template for your system.

REAGENTS

Contents

Each kit contains the following items:
Two HDLD Reagent Cartridges (2 x 200 tests)

Volume per Test	
Sample Volume	3 µL
Total Reagent Volume	280 µL
Cartridge Volumes	A 210 µL B 70 µL C – –

Reactive Ingredients	
Cholesterol esterase (Pseudomonas)	375 U/L
Cholesterol oxidase (E.coli)	750 U/L
Peroxidase (horseradish)	975 IU/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%

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 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.

CALIBRATION

Calibrator Required

SYNCHRON® Systems HDL Calibrator

Calibrator Preparation

1. Use deionized water as the Level 1 calibrator.

2. Remove the metal cap around the HDL calibrator bottle and gently tap the bottle on the table to remove powder at the top of the stopper. Open the HDL calibrator bottle carefully, avoiding loss of lyophilizate.

3. 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.

4. Gently invert until the contents are dissolved avoiding the formation of foam. DO NOT SHAKE.

Calibrator Storage and Stability

The unopened, UniCel SYNCHRON Systems HDL Calibrator may be stored at +2ºC to +8ºC until the expiration date printed on the label. Reconstituted calibrators that are resealed are stable for 14 days at +2ºC to +8ºC or for 30 days at ≤ -20ºC unless the expiration date is exceeded.9 Frozen calibrator should be thawed only once.
Visible signs of microbial growth, gross turbidity, or precipitate in the calibrator may indicate degradation and warrant discontinuation of use.

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 HDL assay must be calibrated every 28 days or with each new cartridge of reagent and also with certain parts replacements or maintenance procedures, as defined in the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual. This assay has within-lot calibration available. Refer to the UniCel DxC 600/800 Systems *Instructions For Use* (IFU) manual for information on this feature. The within-lot calibration option allows loading of a reagent cartridge of the same lot number without the need for recalibration up to and including 90 days. Refer to the UniCel DxC 600/800 System *Instruction for Use* manual for information on this feature.

3. For detailed calibration instructions, refer to 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 UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

Traceability

For Traceability information refer to the Calibrator instructions for use.

QUALITY CONTROL

See Related Documents J-F-CH0820 DXC 800 Controls

STEPS

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 operation. To load samples manually refer to the FHS DXC Series Manual Sample Programming procedure. .For detailed testing procedures, refer to the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual

CALCULATIONS

SYNCHRON® System(s) 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.

ANTICOAGULANT TEST RESULTS

The following anticoagulants were assessed by Deming regression analysis with a minimum of 60 paired serum and plasma samples. Values of serum (X) ranging from 17 mg/dL to 127 mg/dL were compared with the values for plasma (Y) yield the following results.

Anticoagulant	Level of Anticoagulant Tested	Deming Regression Analysis
Lithium Heparin	17 Units/mL	Y = 1.012X + 0.7; r = 0.998
Sodium Heparin	17 Units/mL	Y = 1.030X - 0.3; r = 0.997

PERFORMANCE CHARACTERISTICS

Reference Range

Cardiovascular Risk low	>= 60mg/dL
Within Normal Limits	40 – 90 mg/dL
Cardivascular Risk High	< 40 mg/dL

Analytic Range

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

Sample Type	Conventional Units
Serum or Plasma	5 – 135 mg/dL

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

Reporting results outside of analytical range

Lower limit of detection	5 mg/dL	Results below 5, report as <5 mg/dL
Upper limit of detection	135 mg/dL	Results >135 should be diluted with 0.9% saline, reanalyzed and dilution factor applied. The maximum allowable dilution is X2. Results >270 are reported as >270 mg/dL.

Sensitivity

Limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) data analysis was performed in accordance with the CLSI EP17-A2 guideline.21 The LoB corresponds to the concentration below which analyte-free samples are found with 95% confidence. The LoD corresponds to the sample concentration above the LoB which is detectable with 95% confidence. The LoQ is defined as the lowest amount of analyte in the sample that can be quantitatively determined with stated acceptable precision and trueness, under stated experimental conditions. A properly operating UniCel DxC 600/800 System should exhibit detection limit values equal to the following:

The detection limit results using serum samples support the LoB, LoD, and LoQ specifications in the table below using the HDL reagent on a UniCel DxC 600/800 System.

LIMITATIONS

None identified.

Interferences

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

Substance	Source	Level Tested	Observed Effect
Hemoglobin	RBC hemolysate	500 mg/dL INDEX of 10	No significant interference (within 10%)
Bilirubin	Mixed Isomers	40 mg/dL INDEX of 20	No significant interference (within 10%)
Triglyceride	Human	900 mg/dL	No significant interference (within 10%)
Lipemia	Intralipid	1500 mg/dL INDEX of 10	No significant interference (within 10%)
Ascorbic Acid	NA	20 mg/dL	No significant interference (within 10%)
Immunoglobulin IgG	Human	5000 mg/dL	No significant interference (within 10%)

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 patient samples with elevated serum immunoglobulin levels. In very rare cases gammopathy, especially monoclonal IgM (Waldeström's macroglobulinemia), may cause unreliable results.

4. Falsely low results may be obtained in patients with Type III hyperlipidemia.

5. Refer to References for other interferences caused by drugs, disease and preanalytical variables.

ADDITIONAL INFORMATION

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

REFERENCES

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DOCUMENT APPROVAL **Purpose of Document / Reason for Change:**			
Formatting, name of test changed from HDLD to HDL (this is a Beckman name change), changed clinical significance, methodology and chemical reaction based on latest info from Beckman (procedure from July 2015), change in reactive ingredients, change in calibrator, preparation, storage and stability, change in sensitivity, added max dilution, change in limitations.			
☐ *No significant change to process in above revision. Per CAP, this revision does not require further Medical Director approval.*			
Committee Approval Date	☐ Date: ☒ N/A – revision of department-specific document which is used at only one facility	**Medical Director Approval** *(Electronic Signature)*	Katie Wilkinson, MD 9/25/15