HDL-CHOLESTEROL

OSR6195	**4 x 30 mL**	**R1**
	4 x 10 mL	**R2**
OSR6295	**4 x 50 mL**	**R1**
	4 x 16.5 mL	**R2**

Intended Use
System reagent for the quantitative determination of HDL-Cholesterol concentrations in human serum and plasma on Beckman Coulter AU analyzers.

Summary
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]

Under the guidelines issued by The National Cholesterol Education Program Adult Treatment Panel 2 (NCEP ATP 2),[4] it is recommended that both HDL-Cholesterol and Total Cholesterol should be measured in the initial screening for hypercholesterolemia.
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
The 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- lipoproteins. The HDL cholesterol is released for reaction with cholesterol esterase, cholesterol oxidase and a chromogen system to yield a blue color complex which can be measured bichromatically at 600/700nm. The resulting increase in absorbance is directly proportional to the HDL-C concentration in the sample.

Reaction Phase 1

$$\text{LDL, VLDL Chylomicrons} \xrightarrow[\text{DSBmT + Peroxidase}]{\text{Accelerator + CO}} \text{Colorless end product}$$

Reaction Phase 2

$$\text{HDL Cholesterol} \xrightarrow{\text{HDL Specific detergent}} \text{HDL Disrupted}$$

$$\text{HDL Cholesterol + } H_2O + O_2 \xrightarrow{\text{CHE and CHO}} \text{Cholest-4-en-3-one + } H_2O_2$$

$$H_2O_2 + \text{DSBmT + 4-AAP} \xrightarrow{\text{Peroxidase}} \text{Blue color complex}$$

This reagent was tested in a Cholesterol Reference Method Laboratory Network (CRMLN) laboratory to confirm that it meets the guidelines of the NCEP.

System Information
For AU400/400^e/480, AU600/640/640^e/680 and AU2700/5400/5800 Beckman Coulter Analyzers.

Reagents
Final concentration of reactive ingredients:

Goods Buffer (pH 6.0)		
Cholesterol esterase (Pseudomonas)	375	U/L
Cholesterol oxidase (E.coli)	750	U/L
Peroxidase (Horseradish)	975	U/L
Ascorbate oxidase (Curcubita sp,)	2250	U/L
DSBmT	0.75	mmol/L
4-aminoantipyrine	0.25	mmol/L
Detergent	0.375	%
Preservative	0.05	%

Precautions
1. For *in vitro* diagnostic use.
2. Do not ingest. Harmful if swallowed.

Preparation of Reagents
The HDL-Cholesterol reagent is ready for use. No preparation is required.

Storage and Stability
1. The unopened reagents are stable until the expiration date printed on the label when stored at 2 – 8°C.
2. Opened reagents are stable for 30 days when stored in the refrigerated compartment of the analyzer.
3. Do not use reagents that have been frozen.
4. Protect the reagents from direct sunlight.

HDL-Cholesterol

Indications of Deterioration
Visible signs of microbial growth, turbidity, or precipitate in the HDL-Cholesterol reagent may indicate degradation and warrant discontinuance of use.

Specimen Collection and Preparation

Serum and EDTA or heparinized plasma samples drawn from the patient after a 12 – 14 hour fast are the recommended specimens. Separate serum from red blood cells as soon as possible (within 3 hours). Plasma using anticoagulants such as citrate or oxalate are not recommended.[6]

Sample Storage and Stability
Use fresh sample for analysis when possible. Serum or plasma should not remain at 15 – 30°C longer than 14 hours. If analysis is not completed within 14 hours serum or plasma may be stored at 2 – 8°C for up to 1 week. If specimens need to be stored for more than 1 week they may be preserved at less than - 70°C for up to 3 months. Samples should only be frozen once.[6]

Interfering Substances
Results of studies conducted[7] show that the following substances interfere with this HDL-Cholesterol procedure.

The criteria for no significant interference is recovery within 10% of the initial value.

Ascorbate:	No significant interference up to 20 mg/dL Ascorbate
Bilirubin:	No significant interference up to 40 mg/dL conjugated Bilirubin
	No significant interference up to 40 mg/dL unconjugated Bilirubin
Globulin	No significant interference up to 5 g/dL added Gamma-Globulin
Hemolysis:	No significant interference up to 500 mg/dL Hemolysate
Lipemia:	No significant interference up to 1500 mg/dL Intralipid*
Triglyceride:	No significant interference up to 900 mg/dL Triglyceride**

*Intralipid,® manufactured by KabiVitrium Inc., is a 20% IV fat emulsion used to emulate extremely turbid samples.
** Triglyceride concentrate, manufactured by Miles Pentex, cat. no. 96-051-6, was used to measure triglyceride interference. No significant interference was observed from samples containing native triglycerides up to 1000mg/dL. However, there is poor correlation between lipemia and triglyceride concentration. Triglyceride inference not tested on the AU5800 analyzer.

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc. makes no representation about the completeness or accuracy of results generated by future studies. For further information on interfering substances, refer to Young[8] for a compilation of reported interferences with this test.
In very rare cases gammopathy, especially monoclonal IgM (Waldeström's macroglobulinemia), may cause unreliable results.

Procedure

A complete list of test parameters and operational procedure can be found in the User's Guide appropriate to the analyzer.

Materials Provided
HDL-Cholesterol Reagent

Materials Required But Not Provided
HDL-Cholesterol Calibrator (Cat # ODC0023)

Stability of Final Reaction Mixture
The Beckman Coulter AU analyzer automatically computes every determination at the same time interval.

Calibration
The frequency of calibration is every 7 days. Calibration of this HDL-Cholesterol procedure is accomplished by the use of the HDL-Cholesterol Calibrator material (Cat. No. ODC0023).

Recalibration of this test is required when any of these conditions exist:
1. A reagent lot number has changed or there is an observed shift in control values.
2. Major preventative maintenance was performed on the analyzer.
3. A critical part was replaced.

Quality Control
During operation of the Beckman Coulter AU analyzer, at least two levels of lipid control material should be tested a minimum of once a day. In addition, controls should be performed after calibration, blanking, with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Results
No manual calculations are required. Results are printed out automatically for each sample in mg/dL at 37°C. For S.I. Units (mmol/L) the results must be divided by 38.7.

Dynamic Range

The HDL-Cholesterol procedure is linear from 2.5 mg/dL to 200.0 mg/dL. Samples should be diluted with physiological saline, the assay repeated and the result multiplied by the dilution factor whenever values exceed the upper limit of linearity. Endogenous triglyceride levels gave acceptable performance up to 2000 mg/dL. Samples with triglyceride levels > 2000 mg/dL should be diluted. Samples may be diluted, repeated, and multiplied by the dilution factor automatically utilizing AUTO REPEAT RUN.

Expected Values

Adults:[9] 23 – 92 mg/dL
Expected values may vary with age, sex, diet, and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Specific Performance Characteristics

The following data was obtained using the HDL-Cholesterol Reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may differ.

Precision[11]

Estimates of precision, based on CLSI recommendations[10], are consistent with typical performance. The within run precision is less than 3% CV and total precision is less than 5% CV. Assays of control sera were carried out and data reduced following CLSI guidelines.

N = 80	Within run		Total	
Mean, mg/dL	SD	CV%	SD	CV%
38.85	0.17	0.43	1.03	2.64
66.39	0.39	0.59	1.45	2.19
86.33	0.52	0.60	1.83	2.12

Method Comparison[11]

Patient samples were used to compare this HDL-Cholesterol Reagent. The table below demonstrates representative performance on the AU analyzers.

Y Method	AU640
X Method	Method 2
Slope	1.07
Intercept	-1.2
Correlation Coeff. (r)	0.991
No. of Samples (n)	115
Range (mg/dL)	24 – 89

Sensitivity

Typical change in absorbency for 1 mg/dL of HDL-Cholesterol is 1 mA.

Note

Carryover from this HDL Cholesterol reagent to Lipase reagent may result in elevated lipase values. Please refer to the User Update “Special Parameters – HDL/LDL Cholesterol Carryover Prevention” for proper programming instructions for your AU system.

References

1. NIH Consensus Conference: Triglyceride, High-density Lipoprotein, and Coronary Heart Disease. JAMA 1993; 269: 505-10.
2. Wiebe, D.A, Warnick, G.R., Measurement of High-density Lipoprotein Cholesterol Concentration. In: Rifai N., Warnick G.R., eds. Laboratory Measurement of Lipids, Lipoproteins and Apolipoproteins. Washington, DC: AACC Press, 1994: 91-105.
3. Badimon, U.U., *et al.* Regression of atherosclerotic lesions by high-density lipoprotein plasma fraction in the cholesterol-fed rabbit. J. Clin. Invest. 1990; 85: 1234-1241.
4. Warnick, G.R., et al, National Cholesterol Education Program recommendations for Measurement of High Density Lipoprotein Cholesterol: Executive Summary Clin. Chem. 1995; 41: 1427 - 1433.
5. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III). The Expert Panel, JAMA 2001; 285: 2486-97.
6. Tietz, Textbook of Clinical Chemistry, 3rd Edition, W.B. Saunders, 1999, 849 .
7. CLSI/NCCLS, Interference Testing in Clinical Chemistry EP7-A, 2002.
8. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 5th Edition, AACC Press, 2000.
9. Beckman Coulter Inc. data on samples collected from 120 blood donors in Texas.
10. CLSI/NCCLS Evaluation of Precision Performance of Clinical Chemistry Devices, EP05-A, 1999.
11. Data is on file for specific AU analyzers.

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