Direct HDL/LDL-Cholesterol Calibrator Procedure No. AX158

For the Calibration of HDL-Cholesterol and LDL-Cholesterol LiquiColor® Reagent Sets #BQ0371.

Summary and Principle

Lipoproteins are spherical-shaped particles that contain varying amounts of cholesterol, triglycerides, phospholipids and proteins. The phospholipids and proteins make up the outer surface of the lipoprotein particle, while the core consists mostly of cholesterol in esterified form and triglycerides. The purpose of the lipoprotein particles is to transport cholesterol and triglyceride through the bloodstream.

The relative amounts of the protein and lipid constituents determine the density of the lipoprotein particles and provide a basis for their classification[1]. These classes are: very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins HDL). There have been many clinical studies that have shown that these lipoprotein particles have very distinct and varied effects on the risk of coronary heart disease (CHD)[2-4]. The studies all point to LDL cholesterol as the key factor in the pathogenesis of artherosclerosis and coronary artery disease (CAD)[2-8], while HDL cholesterol has often been observed to have a protective effect.

Both the Direct HDL Cholesterol and Direct LDL Cholesterol LiquiColor® reagent sets provide simple and effective means for determining levels of HDL and LDL cholesterol. This serum calibrator has been developed to provide accurate calibration of both the Direct HDL and Direct LDL Cholesterol LiquiColor® Assays #BQ0371.

Reagents

Direct HDL/LDL Cholesterol Calibrator, Cat No. AX1580

Preparation of lyophilized human serum containing lipoproteins from the various lipoprotein classes including high-density lipoproteins (HDL) and low-density lipoproteins (LDL).

Precautions: *For In Vitro Diagnostic Use Only.* Do not pipette by mouth. NOTE: This calibrator was not tested or certified by the CRMLN (Cholesterol Reference Method Laboratory Network)

Warning: Human source material. Treat as potentially infectious. Each plasma donor unit used in the preparation of this product has been tested by an FDA approved method and found non-reactive for the presence of HBsAg, HCV, and antibodies to HIV 1/2. Because no known test method can offer complete assurance that hepatitis B virus, Human Immunodeficiency Virus (HIV) or other infectious agents are absent, all human-based products should be handled in accordance with good laboratory practices using appropriate precautions. All material used in this test should be considered potentially infectious. Universal precautions as they apply to your facility should be used for handling and disposal of materials during and after testing. Do not use calibrator after the expiration date printed on the label.

Preparation: Reconstitute lyophilized serum calibrator with 3.0 mL of reagent grade water. Close the vial and let stand for 5 minutes at room temperature (15 -30 °C). Swirl gently, avoiding the formation of foam. **Do Not Shake !**

Storage and Stability: The unopened calibrator is stable stored at 2-8°C until expiration date on the label. Once opened and reconstituted the calibrator is stable for 21 days at 2-8°C. Contamination must be avoided. Reconstituted stability of the calibrator may be extended by aliquoting and freezing the reconstituted calibrator preparation at -80°C. Presence of extreme turbidity or growth may indicate deterioration.

Materials Required But Not Provided

Direct HDL-Cholesterol LiquiColor® reagent set, Cat. No. AX1540• Direct LDL-Cholesterol LiquiColor® reagent set, Cat. No. BQ0371• Automated Chemistry Analyzer capable of utilizing a two-reagent system • Pipette to accurately deliver 3.0 mL • Reagent grade water

Automated Analyzers

Below is a general example of the Direct HDL Cholesterol LiquiColor® test procedure for an automated analyzer. All analyzer applications should be validated in accordance with NCEP and CLIA recommendations[9].

		37°C		37°C	
Sample +	Reagent 1		Reagent 2		Measurement (abs. difference
3 µL	300 µL	5 min.	100 µL	5 min.	between 700nm & 600 nm)

HDL – C result

Calibration: The use of the Direct HDL/LDL Calibrator is required for calibration of the Direct HDL & Direct LDL Cholesterol LiquiColor® assays. If control results are found to be out of range, the procedure should be recalibrated. The instrument manufacturer's calibration guidelines should be followed to calibrate your analyzer. The values of HDL and LDL cholesterol in the calibrator are found on the label and are specific for each lot of product. Make sure that the values entered into the auto analyzer matches the values on the calibrator vial label for HDL and LDL.

Expected Values

The expected values for serum HDL cholesterol are as follows:

Males: 30 - 70 mg/dL
Females: 30 - 85 mg/dL

According to the NCEP, HDL values greater than or equal to 40 mg/dL are considered desirable and values greater than or equal to 60 mg/dL are considered to offer some protection against coronary heart disease. Values below 40 mg/dL are considered to be a significant independent risk factor for coronary heart disease[8].

The following NCEP recommendations for patient classifications are suggested for the prevention and management of coronary heart disease[8].

LDL Cholesterol	Classification
< 100 mg/dL (< 2.58 mmol/L)	Desirable
130 - 159 mg/dL (3.36-4.11 mmol/L	Borderline High Risk
160-189 mg/dL (4.14-4.88 mmol/L)	High Risk
> 190 mg/dL (> 4.90 mmol/L)	Very High Risk

Performance Characteristics[13]

Data was derived on Hitachi® 917 analyzer using Direct HDL Reagent, AX1540

Accuracy: Linear regression analysis of 50 serum samples with HDL cholesterol levels ranging from 18 to 123 mg/dL was performed, comparing the present method (y) to a commercially available direct HDL method (x) with the following results: y = 1.01x - 0.4942, r = 0.9987. (Studies were performed according to NCCLS Guideline, EP9-T)

Precision: Within-Day and Day-to-Day precision for the Direct HDL Cholesterol LiquiColor® method was determined following a modification of NCCLS document EP5-T2. Precision studies produced the following results

	Mean (mg/dL)	SD	CV%
Within-Day	38	0.24	0.65
	74	0.42	0.56
Day-to-Day	**Mean (mg/dL)**	**SD**	**CV%**
	32	0.64	2.0
	47	0.76	1.6
	68	1.22	1.8

Sensitivity: Based on an instrument resolution of A=0.001 absorbance units, this reagent has a sensitivity of 0.4 mg/dL of HDL cholesterol.

Linearity: When performed as directed this method is linear to 200 mg/ dL. Performed according to NCCLS Guideline EP6-P.

Data was derived on Hitachi® 917 analyzer using Direct LDL Reagent, BQ0371

Accuracy: Linear regression analysis of 62 serum samples with LDL cholesterol levels ranging from 22 to 178 mg/dL was performed, comparing the present method (y) to a commercially available direct LDL method (x) with the following results: y = 1.025x - 4.0289, r = 0.9969(Studies performed according to NCCLS Guideline, EP9-T)

Precision: Within-Day and Day-to-Day precision for the Direct LDL Cholesterol LiquiColor® method was determined following a modification of NCCLS document EP5-T2. Precision studies produced the following results:

	Mean (mg/dL)	SD	CV%
Within-Day	50	0.28	0.56
	99	0.43	0.44
	Mean (mg/dL)	**SD**	**CV%**
	97	1.29	1.33
Day-to-Day	138	1.92	1.40
	205	2.90	1.43

Sensitivity: Based on an instrument resolution of A=0.001 absorbance units, the method presented shows a sensitivity of 0.4 mg/dL of LDL Cholesterol.

Linearity: When performed as directed this method is linear to 520 mg/dL. Performed according to NCCLS Guideline, EP6-P.

References

1. Gotto A.M., Lipoprotein metabolism and the etiology of hyperlipidemia,Hospital practice, 23: Suppl. 1,4 (1988).
2. Crouse J.R. et al., Studies of low density lipoprotein molecular weight in human beings with coronary artery disease, J. Lipid Res., 26;566 (1985).
3. Kannel W.B., Castelli W.P., Gordon T., Cholesterol in the Prediction ofArteroscl erotic Disease; New Perspectives Based on the Framingham Study, Am. Intern. Med., 90; 85 (1979)
4. Kannel W.B., et al., Optima; Resources for Primary Prevention of Artherosclerotic Disease, Circulation, 70;157A-205A, (1984).
5. Schaefer E.J., et al., Pathogenisis and Management of Lipoprotein Disorders, N. Eng. I. Med., 312 (20) 1300-1310 (1985).
6. The Lipid Research Clinics, Coronary Primary Prevention Trial Results., JAMA, 251 (3) 351-374 (1985).
7. Consensus Development Conference: Lowering Blood Cholesterol to Prevent Coronary Heart Disease, JAMA, 253 (14), 2080-2086 (1985).
8. NIH publication No. 01-3670, 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), May 2001.
9. Tietz N.W., Clinical Guide to Laboratory Tests, W.B. Saunders Co., Philidelphia, p. 256 (1986).

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