Direct LDL-Cholesterol LiquiColor® Procedure No. 0710

For the Quantitative Determination of Low Density Lipoprotein (LDL) Cholesterol in Human Serum or Plasma.

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 these apolipoproteins determine the density of the lipoprotein particles and provide a basis for their classification. 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). High LDL-C levels have repeatedly been associated with an increased risk of coronary heart disease and coronary artery disease. Thus, the determination of serum LDL cholesterol has been recognized as a useful tool in identifying high-risk patients.

Historically, most laboratories have used the Friedwald equation to calculate the LDL cholesterol based on results from 3 separate assays:

- Total cholesterol
- HDL cholesterol
- Triglycerides

This equation has limitations including the fact that the triglyceride value cannot exceed 400 mg/dL in the sample. As a result, the routine determination of LDL-C has suffered from both long turnaround times and poor reproducibility.

The Stanbio Direct LDL Cholesterol LiquiColor® is a homogenous method for directly measuring serum LDL-C levels without the need for any off-line pretreatment or centrifugation steps. The method employs a two-reactant system. The first reagent (R1) contains a combination of detergent, organic and inorganic phosphoric acid compounds which specifically binds HDL, VLDL and chylomicrons leaving the LDL particles exposed. The second reagent (R2) contains enzymes which then react with the LDL cholesterol present in the sample. Consequently, only the LDL cholesterol is subject to cholesterol measurement.

Reagents

**Direct LDL Cholesterol LiquiColor® Buffer (R1), Cat. No. 0711**
- Magnesium Sulfate: 2.5 mmol/L
- HDAOS: 0.8 g/L
- Good's Buffer (pH 7.0 ± 0.1)
- Stabilizers, detergents and preservatives.

**Direct LDL Cholesterol LiquiColor® Enzyme (R2), Cat. No. 0712**
- Cholesterol Oxidase: > 5,000 U/L
- Cholesterol Esterase: > 800 U/L
- Peroxidase: > 15,000 U/L
- 4-aminoantipyrine: 0.5 mmol/L
- Good's Buffer (pH 6.8 ± 0.1)

**Precautions:** For In Vitro Diagnostic Use Only. Do not pipette by mouth. All specimens 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 reagents after the expiration date printed on their respective labeling.

**Materials Required But Not Provided:**
- Stanbio Direct HDL/LDL-Cholesterol Calibrator, Cat. No. 0595
- Automated Chemistry Analyzer capable of utilizing a two-reagent system

**Expected Values**

The following NCEP recommendations for patients classifications are suggested for the prevention and management of coronary heart disease:
- Desirable: ≤ 100 mg/dL (≤ 2.58 mmol/L)
- Borderline High Risk: 130 - 159 mg/dL (3.36 - 4.11 mmol/L)
- High Risk: 160 - 189 mg/dL (4.14 - 4.88 mmol/L)
- Very High Risk: ≥ 190 mg/dL (≥ 4.90 mmol/L)

It is recommended that each laboratory establish its own range of expected values, since differences exist between instruments, laboratories, and local populations.

**Performance Characteristics**

Data was derived on Hitachi® 917 analyzer.

**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 HDL Cholesterol LiquiColor® method was determined following a modification of NCCLS document EP5-T. Precision studies produced the following results:

- Within-Day
  - Mean (mg/dL): 50, 99
  - SD: 0.28, 0.43
  - CV%: 0.56, 0.44
- Day-to-Day
  - Mean (mg/dL): 97, 204
  - SD: 1.29, 2.90
  - CV%: 1.33, 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**

12. Stanbio Laboratory Data

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