# HDL-c Direct (Homogeneous.)

**INTENDED USE**
Bioline HDL is used for the quantitative determination of high density lipoprotein (HDL) in human serum.

**CLINICAL SIGNIFICANCE**
Cholesterol is a fatty substance found in blood, bile and brain tissue .It serves as a precursor to bile acids, steroids and vitamin D. The concentration of total cholesterol in serum has been associated with metabolic, infectious and coronary heart diseases .In the plasma ,cholesterol is transported by three lipoproteins: high density lipoprotein(HDL-Cholesterol) ,low density lipoprotein (LDLCholesterol),and very low density lipoprotein (VLDL-Cholesterol). Castelli and co-workers have indicated that an inverse relationship exists between serum HDL-Cholesterol and the risk of coronary heart disease. The measurement of total and HDL Cholesterol and triglyceride provides valuable information for the prediction of coronary heart disease and for lipoprotein phenotyping.

**METHOD AND PRINCIPLE**
The method is based on a modification of classic co precipitation methods using optimized amounts of polyvinyl sulfonic acid (PVS) and polyethylene glycol methyl ether (PEGME) associated to selective surfactants. On the first step of the reaction, LDL, VLDL, and chylomicrons (CM) react with PVS and PEGME, which makes them inaccessible to cholesterol oxidase (CHOD) and cholesterol esterase (CHER). After the addition of reagent 2, CHOD and CHER react specifically with HDL in sample, yielding $H_2O_2$, which is detected by the Trinder reaction. The color intensity is directly proportional to the concentration of HDL cholesterol in sample.

PVS / PEGME
HDL+LDL+VLDL+CM ---------- HDL+(LDL+VLDL+CM)

HDL+CHOD+CHER ---------- Fatty acid +H2O2

2H2O2+4AAP+TODB -------- Quinoneimine +5H2O

**REAGENT COMPOSITION**
**Reagent 1 :** MES buffer pH 6.5 10 mM; TODB N,N-bis(4-sulfobutyl) -3-methylaniline 1 mM; polyvinyl sulfonic acid 2mg/L; polyethylene glycol methyl ether 2g/L; magnesium chloride 1.6g/l; Surfactant 0.5g/l; EDTA 1.0 g/l and preservative.

**Reagent 2 :** MES buffer pH 6.5 10 mM; cholesterol esterase 4000 U/L; cholesterol oxidase 10000 U/L; 4-aminoantipyrine 2.5 mM; peroxidase 30000 U/L; EDTA 1.0 g/l; preservative; and surfactant.

**Calibrator - Lyophilized reagent. Store at 2 - 8 °C.**
Check the calibrator concentration on the bottle label. Preparation containing human HDL cholesterol and preservative 1%. After reconstitution, the calibrator is stable for 7 days at 2 - 8 ºC and 30 days at -20 ºC.

The calibration is valid only for the reagents and calibrator from the same lot number. **Reagents and calibrators are not interchangeable between kits with different lot numbers**.

**WARNINGS AND PRECAUTIONS**
1. For *in-vitro* diagnostic use only.
2. Exercise the normal precautions required for the handling of all laboratory reagents .Pipetting by mouth is not recommended for any laboratory reagent.
3. The calibrator contains human blood derivatives and was tested for the presence of HBsAg and anti-HCV and anti-HIV antibodies with approved tests, yielding negative results for all of them. However, no known test method can offer complete assurance that human blood samples will not transmit infectious diseases. Therefore, all blood derivatives should be considered potentially infectious.

**REAGENT PREPARATION**
Reagent is ready to use as supplied. Unopened reagents, when stored at indicated temperature, are stable up to the expiration date shown on the label.

**REAGENT AND CALIBRATOR STORAGE AND STABILITY**
Unopened reagents, when stored at indicated temperature, are stable up to the expiration date shown on the label.
The reconstituted calibrator is stable for 7 days at 2 - 8 ºC and 30 days at
-20 ºC when stored in a tube proper for freezing, which avoids solvent evaporation. To avoid repeated freeze and thaw cycles, we recommend dividing the calibrator in aliquots before freezing. When aliquots are thawed, they must be homogenized thoroughly prior to being used. **Do not use vortexing system or similar equipment.** Once they are thawed, do not freeze the aliquots again.

**REAGENT DETERIORATION**
The reagent should be discarded if:
1. Sediment turbidity has occurred.
2. The reagent does not meet stated performance parameters.

**SPECIMEN COLLECTION AND STORAGE**
1. Test specimens should be preferably serum or EDTA Plasma and free from hemolysis.
2. Serum or plasma samples must not be kept at room temperature (15 - 30 ºC) for more than 14 hours. If it is not possible to perform tests within 14 hours after sample collection, samples can be stored at 2 - 8 ºC for up to 7 days and at -20 ºC for up to 30 days. If samples must be stored for longer periods, they must be kept at -70 ºC.
3. Avoid repeated freeze and thaw cycles. Thawed samples must be homogenized thoroughly prior to being tested. Do not use vortexing system or similar equipment. Do not use samples with signs of microbial contamination.

**ASSAY PROCEDURE FOR SEMIAUTO ANALYZER**
Wavelength : 578(546-600) nm.
Temperature : 37°C

| | Blank | Calibrator | Sample |
|---|---|---|---|
| **Reagent 1** | 0.750 mL | 0.750 mL | 0.750 mL |
| **Calibrator** | - | 10 µL | - |
| **Sample** | - | - | 10 µL |

Mix and incubate at 37ºC for 5 min .

| | | | |
|---|---|---|---|
| **Reagent 2** | 0.250 ml | 0.250 ml | 0.250 ml. |

Mix and incubate at 37ºC for 5 min . Read the absorbance of calibrator and sample against reagent blank at 578 nm.

**Calculation:**
HDL mg/dl = $\frac{\text{Abs of sample- Abs of blank X Cal concn}}{\text{Abs of Calibrator-Abs of blank}}$

**LIMITATIONS**
Unconjugated bilirubin up to 40 mg/dL, conjugated bilirubin up to 30 mg/dL, hemoglobin up to 1000 mg/dL, ascorbic acid up to 10 mM and triglycerides up to 400 mg/dL do not interfere

significantly in the reaction.

For samples above the linearity of 185 mg/dl rerun after dilution. Multiply the result with dilution factor to get correct result.

**QUALITY CONTROL**

It is recommended that controls be included in each set of assays. Commercially available control material the established HDL cholesterol values may be used for quality control. The assigned value of the control material must be confirmed by the chosen application. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction, or procedural errors.

**EXPECTED VALUES**

Male HDL : 35-65 mg/dl
Female HDL: 35-80 mg/dl

**PERFORMANCE CHARACTERISTICS**

**Method Comparison**

Comparison studies were carried out using another similar commercially available HDL reagent as a reference. Serum samples were assayed in parallel and the results compared by least squares regression.

The results obtained using 50 samples were the following: Correlation coefficient: 0.938

Regression equation: y= 0.9825x + 1.41606

**Precision study : Within Run**

| | Sample 1 | Sample 2 |
|---|---|---|
| Mean (mg/dL) | 46.5 | 35.4 |
| SD | 2.2 | 2.4 |
| CV% | 3.3 | 4.8 |

**Precision: Run to Run**

| | Sample 1 | Sample 2 |
|---|---|---|
| Mean (mg/dL) | 47.9 | 35.7 |
| SD | 2.4 | 2.8 |
| CV% | 3.5 | 4.5 |

**General Technical Parameters**

| | |
|---|---|
| **Mode** | **End Point** |
| **Wavelength (Filter)** | **578 nm** |
| **Reaction Direction / Type** | **Increasing / Positive** |
| **Reagent Blank** | **Yes** |
| **Sample Vol.** | **10 µL** |
| **Reagent Vol.** | **1000 µL** |
| **Reagent Blank Abs (Max.)** | **< 0.100 Abs** |
| **Incubation time** | **5 min** |
| **Calibration Method** | **1 - Point** |
| **Factor** | **NA (Calculated by analyzer)** |
| **Standard /Calibrator (Conc.)** | **Refer Calibrator vial** |
| **Linearity** | **185 mg/dL** |
| **Decimal Places** | **0** |
| **Temp.** | **37 °C** |
| **Unit** | **mg/dL** |
| **Ref. Low Male/Female** | **35/35 mg/dL** |
| **Ref. High Male/Female** | **65/80 mg/dL** |

**REFERENCES**


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