

# Automated Procedure

**Cat. No. 20230** R1 1 x 45 ml  
R2 1 x 15 ml

**Cat. No. 20231** R1 2 x 45 ml  
R2 2 x 15 ml

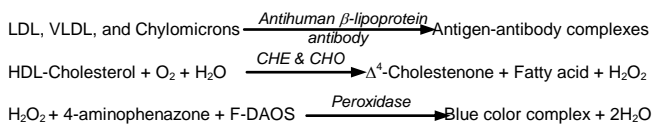
## HDL-Cholesterol

Immuno-inhibition method

### Liquid Reagents

#### Test Principle

Anti human  $\beta$ -lipoprotein antibody in Reagent 1 binds with lipoproteins (LDL, VLDL, and chylomicrons) other than HDL. The antigen-antibody complexes formed block enzyme reactions when Reagent 2 is added. Cholesterol esterase (CHE) and cholesterol oxidase (CHO) in Reagent 2 react only with HDL-C. Hydrogen peroxide produced by the enzyme reactions with HDL-C yields a blue color complex upon oxidative condensation of F-DAOS [N-ethyl-N-(2-hydroxy-3-sulfo-propyl)-3,5-dimethoxy-4-fluoroaniline, sodium salt] and 4-aminoantipyrine (4-AAP) in the presence of peroxidase (POD). By measuring the absorbance of the blue color complex produced, at the optimum wavelength of 593 nm, the HDL-C concentration in the sample can be calculated when compared with the absorbance of the HDL-C calibrator.



#### Concentrations in the test

<b>Reagent R1</b>		
Buffer pH = 7.0	30.0	mmol/L
POD (Peroxidase)	2400	U/L
Ascorbate oxidase	2700	U/L
4-Aminophenazone	0.9	mmol/L
Anti human $\beta$ -lipoprotein antibody		
Detergent, stabilizer		
<b>Reagent R2</b>		
Buffer pH = 7.0	30.0	mmol/L
Cholesterol esterase	4000	U/L
Cholesterol oxidase	20000	U/L
F-DAOS	0.8	mmol/L
Stabilizer		

#### Stability of reagents

**Reagents R1** : liquid, ready to use.

**Reagents R2** : liquid, ready to use.

Use reagents R1 & R2 as supplied. Unopened bottles are stable up to expiry date given on the label when stored at +2  $\rightarrow$  +8  $^{\circ}\text{C}$ . After opening the bottles, these solutions are stable for 60 days at 2 - 8  $^{\circ}\text{C}$ .

#### Specimen collection and handling

1. Serum or heparinized plasma.
2. Serum must be separated from the blood clot as soon as possible.
3. It is recommended to measure HDL-C immediately after collection.
4. Patient should be fasting 12 - 14 hours before the sample is taken.

#### Calibrator

lipids HDL/LDL Calibrator Cat. No. 15871

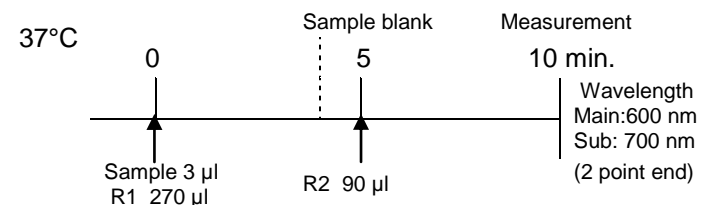
#### Quality control

lipids level 1 Cat. No. 15211

lipids level 2 Cat. No. 15221

A quality control program is recommended for all clinical laboratories. The analysis of control material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. The values obtained for controls should fall within the manufacturer's acceptable ranges. If values are to be established for unassayed control material, the laboratory should assay each level of control material a sufficient number of times to generate a valid mean and acceptable range.

#### Procedure



#### Linearity

Up to 180 mg/dl.

If the result exceeds 180 mg/dl, repeat the test using diluted sample (1+1) with sodium chloride solution (0.9%) and multiply the result by 2.

#### Interferences

1. Ascorbic acid, Bilirubin, and hemoglobin do not have a significant effect on the measurement.
2. Samples with triglyceride concentration exceeding 1200 mg/dl, should be diluted and reanalyzed. Dilute the sample with a saline solution, repeat assay and multiply result by the dilution factor.

#### Performance characteristics

1. Sensitivity :
  - Absorbance of water sample (blank) is 0.1 or less.
  - Absorbance of a 50 mg/dl HDL-Cholesterol sample is 0.07 - 0.34
2. Specificity :
  - Obtained values of control serum samples with known amount of HDL-Cholesterol fall within  $\pm 10\%$ .
3. Precision :
  - Within-run CV of 5 repeated assays is 5% or less.
4. Measurable range :
  - 1 - 180 mg/dl HDL-Cholesterol.

### Precautions

1. The reagent is designed to be used on commercially available automated analyzers. Refer to the operating manual for a description of instrument operation and specifications.
2. Don't use reagent R1 which was frozen by mistake.
3. After opening the reagent, it is not recommended to store it in the instrument for a long period of time. When the opened reagent is stored, cap the bottle and keep it at the specified temperature.
4. Use Medichem HDL-C calibrator (Code No. 15021) for the calibration. Refer to the instruction sheet in the calibrator.
5. Avoid contact with eyes and skin. If contacted, flush eyes or rinse skin with a large amount of water. If irritation persists, consult a physician.
6. When using enzymatic methods for the determination of cholesterol esters, contamination and interference to other clinical chemistry assays on the same instrument in principle cannot be excluded. In the rare event of such a problem occurring, please refer to the instrument's manual for channel setting and washing procedure options.

### Reference range

Total Cholesterol CHOD – PAP	≤ 4 wk.	50 - 170	mg/dl
	2 – 12 mth.	60 - 190	mg/dl
	≥ 1 yr.	110 - 230	mg/dl
	Adults	< 200	mg/dl
HDL- Cholesterol	Adults	> 35	mg/dl
LDL- Cholesterol	Adults	< 155	mg/dl

### References

1. Rifai, N. and Warnick, G.R., Ed. Laboratory Measurement of Lipids, Lipoproteins and Apolipoproteins AACC Press. Washington, DC, USA, 1997.
2. Recommendation of the Second Joint Task Force of European and other Societies on Coronary Prevention. Prevention of coronary heart disease in clinical practice; Eur Heart J 1998;19: 1434-503.
3. Gordon, T., Castelli, W.P., Hjortland, M.C., et al., Am. J. Med. 62, 707-714, (1977).
4. Clinical laboratory diagnostics: Laboratory results /ed. By Lothar Thomas – 1. ed. -Frankfurt/main : TH-books-verl. -Ges., 1998 Einheitsacht.: Labor und Diagnose < engl. > ISBN 3 - 9805215 - 4 - 0 (18) - P : 184.
5. Wiebe DA, Warnick GR. Measurement of high density lipoprotein cholesterol concentration. In: Rifai N, Warnick RG, Dominiczak H, eds. Handbook of lipoprotein testing. Washington: AACC Press, 1997, 12744.