TECO DIAGNOSTICS
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HDL-CHOLESTEROL REAGENT

IVD

INTENDED USE
For the quantitative determination of high density lipoprotein (HDL) in human serum.

INTRODUCTION
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 (LDL-Cholesterol), and very low density lipoprotein (VLDL-Cholesterol).[1]

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.[2, 3]

Our precipitating reagent uses the well established precipitating properties of polyethylene glycol 6000 at pH 10.0. [4, 5, 6]

PRINCIPLE
When serum is reacted with the polyethylene glycol reagent, all the low and very low-density lipoprotein (LDL and VLDL) are precipitated. The HDL fraction remains in the supernatant. The supernatant is then treated as a sample for cholesterol assay.

REAGENT COMPOSITION
1. HDL Cholesterol Precipitating Reagent:
   20% w/v polyethylene glycol 6000 in glycine buffer at pH 10 (25°C). Store at room temperature (15 - 30°C).
2. HDL Cholesterol Standard:
   Cholesterol in alcohol 50 mg/dl. Store tightly capped at room temperature (15 - 30°C).

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.

REAGENT PREPARATION
Reagent is ready to use as supplied.

STORAGE AND STABILITY
Store the reagent set at room temperature (15-30°C) tightly capped. The reagent is stable until the expiration date indicated on the bottle label.

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 serum and free from hemolysis.
2. Patient should be fasting for 12 - 14 hours after eating.
3. HDL in serum is reported stable for seven (7) days at 2 - 8°C and for three months frozen.[7]

MATERIALS REQUIRED BUT NOT PROVIDED
1. Enzymatic Cholesterol Reagent Set
2. Centrifuge
3. Test tubes/rack
4. Timer
5. Heating block
6. Spectrophotometer

AUTOMATED PROCEDURE
Refer to appropriate application manual available.

MANUAL PROCEDURE
1. Label tubes: control, patient, etc.
2. Mix equal amount of serum and HDL cholesterol precipitating reagent in the glass tube and mix vigorously, e.g. 0.2 ml serum + 0.2 ml HDL precipitating reagent.
3. Centrifuge for ten (10) minutes at 1500 - 2000 g. (standard lab centrifuge).
4. Separate supernatant from precipitate. The supernatant fraction contains HDL.

* *TC MULTI-PURPOSE CALIBRATOR MAY BE USED TO REPLACE STANDARD.*

PROCEDURE
Run the total cholesterol assay according to the instructions, but double the sample volume to compensate for the previous dilution. If the total cholesterol test phenol free requires a 0.025 ml (25 µl) sample, use 0.05 ml (50 µl) for the HDL determination. Keep original sample volumes for standards.

NOTE: If the supernatant is cloudy/hazy, the sample should be re-centrifuged. If the sample still remains cloudy, dilute the serum sample 1:1 with saline and start the procedure over. Final results must be multiplied by two (2).

LIMITATIONS
Hemolyzed and icteric specimens should not be used.

QUALITY CONTROL
It is recommended that controls be included in each set of assays. Commercially available control material with 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[2]

Male HDL:	26 - 63 mg/dl
Female HDL:	33 - 75 mg/dl

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

PERFORMANCE CHARACTERISTICS

1. *Comparison:*
   A comparison between this procedure and identical HDL Cholesterol precipitating reagent (commercial product) yields a regression equation of y = 0.99 x - 0.05 with a coefficient of correlation of $R^2 = 0.93$
2. *Precision:*

	Within Run	
Mean (mg/dl)	S.D.	C.V. (%)
46.5	2.4	5.2
35.4	2.4	6.8

	Run-to-Run	
Mean (mg/dl)	S.D.	C.V. (%)
47.9	4.4	9.1
35.7	4.4	12.3

REFERENCES

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H511: 10/2022

Manufactured by:

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