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HDL-CHOLESTEROL REAGENT

HDL CHOLESTEROL PRECIPITATING REAGENT
For the quantitative determination of high density lipoprotein (HDL) in 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 (LDLCholesterol) 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 Cholesterols and triglycerides 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
HDL Cholesterol Precipitating Reagent.
20% w/v polyethylene glycol 6000 in glycine buffer at pH 10 (25°C).
Store at room temperature.
Cholesterol Standard: Cholesterol in alcohol 50mg/dl store at 2 - 8°C tightly capped.

WARNINGS AND PRECAUTIONS
For *In Vitro* Diagnostic Use.

REAGENT PREPARATION
Reagent is ready to use as supplied.

STORAGE AND STABILITY
Store the reagent set at room temperature tightly capped. The reagent is stable as appeared on the label.

REAGENT DETERIORATION
The reagent should be discarded if:
1. Sediment/turbidy 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.

PROCEDURE (AUTOMATED)
Consult the appropriate instrument application guide available from us.

PROCEDURE (MANUAL)
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. 0.2ml serum + 0.2ml HDL precipitating reagent.
3. Centrifuge for ten (10) minutes at 15000 - 2000 g. (standard lab centrifuge).
4. Separate supernatant from precipitate. The supernatant fraction contains HDL.

* USE MULTI PURPOSE CALIBRATOR TO REPLACE STANDARD.

CHOLESTEROL 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 0.01 ml - (10 ul) sample, use 0.02 ml (20 ul) for the HDL determination. Keep original sample volumes for standards.

PROCEDURE NOTES
If the supernatant is cloudy/hazy, the sample should be recentrifuged. 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.

PROCEDURE 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 either reagent deterioration, instrument malfunction, or procedural errors.

EXPECTED VALUES[2]

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

PERFORMANCE CHARACTERISTICS

1. COMPARISON: A comparison between this procedure and identical HDL Cholesterol precipitating reagent (commercial product) yields a regression equation of y = 0.99x - 0.05 with a coefficient of correlation of $R^2 = 0.93$
3. 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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