POINTE SCIENTIFIC, INC.
5449 Research Drive • Canton MI 48188 • USA

HDL Cholesterol Precipitating Reagent Set

Intended Use
For the quantitative determination of High Density Lipoprotein (HDL) in Cholesterol in serum.

Method History
Early methods of HDL determination involved preparative ultracentrifugation.[1] Even though this method has undergone several modifications[2] and is considered the reference method today, it remains a tedious, time consuming procedure requiring expensive equipment and highly trained personnel. Electrophoresis has long been used for separation and qualitative estimation of lipoproteins but has not been used as a quantitative tool due to problems of standardization and poor precision.[3,4,5] The most recent separation methods involved the use of polyanions and divalent cations to precipitate low density lipoproteins leaving the HDL in the supernatant.[6] Some of the reagents used included; Heparin-Mn,[7] Sodium Phosphotungstate-Mg,[8] Dextran Sulfate[9] and others. The above methods displayed certain drawbacks concerning lipemic serums, interferences with enzymatic cholesterol procedures, etc. Vikari described a method utilizing polyethylene glycol 6000 in 1976.[10] This method has come under criticism and has since been revised.[11,6] Izzo et al[12] describe modifications on the original procedure that yield a simple, accurate procedure with values corresponding to the ultracentrifugation method with none of the interferences associated with the other reagents. The present procedure is based on that modification.

Principle
When serum is combined with the polyethylene glycol reagent, all beta-lipoproteins (LDL and VLDL) are precipitated. The HDL fraction (alpha-fraction) remains in the supernatant. The supernatant is then treated as a sample and assayed for cholesterol by an enzymatic method. The value obtained is the HDL cholesterol value.

Reagents
HDL Cholesterol Precipitating Reagent: 20% w/v polyethylene glycol in glycine buffer at pH 10.0 (25°C)

Reagent Preparation
Reagent is ready to use as supplied.

Reagent Storage
Store at room temperature (15-30°C) tightly capped.

Reagent Deterioration
Do not use if:
1. Crystals/sediment appear in the reagent.
2. The reagent does not meet stated performance parameters.

Precautions
This reagent is for *in vitro* diagnostic use only.

Specimen Collection and Storage
1. Fresh, unhemolyzed serum is recommended.
2. Patient should be fasting 12-14 hours before the sample is taken.
3. HDL in serum is reported stable for seven days at 2-8°C and for three months at -20°C.[9]

Materials Provided
HDL Cholesterol Precipitating Reagent.

Materials Required but not Provided
1. Enzymatic Cholesterol Reagent Set
2. Centrifuge capable of 1000-2000g (standard lab centrifuge).
3. Accurate pipetting devices.
4. Timer.
5. Test tubes/rack
6. Heating Block (37°C).
7. Spectrophotometer capable of reading at 500nm.

Procedure
A. Separation of HDL Cholesterol
   1. Label tubes for appropriate controls and patients.
   2. Pipette 0.5 ml (500 ul) sample into respective tubes.
   3. Pipette 0.5 ml (500 ul) reagent into each tube and mix using vortex.
   4. Centrifuge at 1000-2000g for 10 minutes.

B. HDL Cholesterol Determination
   1. Label tubes “Blank”, “Standard”, “Controls”, “Patients”, etc.
   2. Pipette 1.0ml enzymatic cholesterol reagent, prepared according to package insert instructions, into each tube.
   3. Pipette 0.05 ml (50 ul) standard or clear supernatants from step #4 above to respective tubes.
   4. Incubate all tubes for 10 minutes at 37°C.
   5. Zero spectrophotometer at 500 nm with reagent blank.
   6. Read and record absorbances of all tubes at 500 nm.
   7. To obtain values in mg/dl, see “Calculations”.

Procedure Notes
Final HDL results must be multiplied by two to compensate for the sample/precipitating reagent dilution of 1:1. The volume of sample and precipitating reagent may be varied as long as the 1:1 ratio is maintained (e.g. 0.2ml of each).

Limitations
1. Hemolyzed and icteric specimens should not be used since they may falsely elevate the results.
2. Ascorbic acid inhibits the enzymatic cholesterol determination.

Calibration
The test is calibrated with a serum based calibrator or an aqueous cholesterol standard (50mg/dl). Control serums with known HDL values should be run routinely to monitor the validity of the procedure.

Calculations
$$\text{HDL Cholesterol (mg/dl)} = \frac{\text{Abs. Sample}}{\text{Abs. Std}} \times \text{Conc. of Std.} \times 2$$

Where 2 is the dilution factor.

Sample calculation: If Abs. Sample = .100, Abs. Std. = .250, and concentration of Standard = 50 mg/dl then:

$$\frac{0.100}{0.250} \times 50 \times 2 = 40 \text{ mg/dl}$$

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Expected Values[13]

HDL Cholesterol……30-75mg/dl
LDL……66-178mg/dl

LDL can be calculated using the following formula:

$$LDL = \text{Total Cholesterol} - \text{HDL Cholesterol} - \frac{\text{Triglycerides}}{5}$$ [14]

When: Total Cholesterol = 250, HDL = 40, and Triglycerides = 120

Then: $250 - 40 - \frac{120}{5} = 186$ mg/dl LDL

This equation holds true only if the triglycerides value is below 400 mg/dl and the patient does not have type III hyperlipoproteinemia.

Performance

1. Comparison: A study was done between the polyethylene glycol reagent and a Phosphotungstate/Mg method yielding a correlation coefficient of 0.982 with a linear regression equation of y=0.85 x +5.5.
2. Precision:

Within Run			Run to Run		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
37	1.9	5.1	36	1.7	4.7
72	0.9	1.3	73	1.1	1.5

References

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Manufactured by Pointe Scientific, Inc.
5449 Research Drive, Canton, MI 48188

European Authorized Representative:
Obelis s.a.
Boulevard Général Wahis 53
1030 Brussels, BELGIUM
Tel: (32)2.732.59.54 Fax:(32)2.732.60.03 email: mail@obelis.

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