POINTE SCIENTIFIC, INC.
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HDL Cholesterol Precipitating Reagent Set (Dextran Sulfate)

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

Clinical Significance
HDL Cholesterol has been shown to have an inverse correlation with the incidence of cardiovascular disease. Therefore, the determination of HDL is useful in assessing the risk of patients to coronary heart disease. High HDL levels are associated with lower risk and low HDL levels are associated with increased risk.

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.[6-9] The present procedure is based on the recommendations of Warnick et al [10,11] using dextran sulfate and magnesium ions.

Principle
When serum is combined with the reagent, dextran sulfate and magnesium ions precipitate the LDL and VLDL fractions, leaving the HDL fraction in solution. The HDL cholesterol is then determined using an enzymatic cholesterol assay.

Reagents
HDL Cholesterol Precipitating Reagent (concentrations refer to reconstituted reagent): Dextran Sulfate (50,000 MW) 10g/L, Magnesium ions 500mM, Buffer, pH7±0.1, non-reactive ingredients with sodium azide (0.1%) as preservative.

Reagent Preparation
Reconstitute the reagent vial with the volume of distilled water stated on the vial label. Swirl to dissolve.

Reagent Storage
1. Un-reconstituted reagent should be stored at 2-8°C and is stable until the expiration date on the label.
2. Reconstituted reagent is stable for 90 days at 2-8°C.

Reagent Deterioration
Do not use if:
1. There is evidence that moisture has entered the vial, such as caking or incomplete dissolution.
2. The reconstituted reagent is turbid.

Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. This reagent contains sodium azide at 0.1%. This may react with copper or lead plumbing to form explosive metal azides. Upon disposal, flush with large amounts of water to prevent azide build up.

Specimen Collection and Storage
1. Fresh, unhemolyzed serum is recommended.
2. Patient should be fasting 12-14 hours before the sample is taken.
3. Hemolyzed specimens may be used if a sample blank is used to correct for the hemolysis. See “Procedure Notes”.
4. Icteric specimens should not be used.
5. HDL in serum is reported stable for seven days at 2-8°C and for three months at -20°C.[9]

Interferences
1. Hemolysis may cause elevated results. A sample blank is recommended. See “Procedure Notes”.
2. Icteric specimens should not be used.
3. Elevated levels of ascorbic acid inhibit the enzymatic cholesterol determination.
4. For a comprehensive review of interferences see Young et al.[18]

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.
8. Vortex mixer.

Procedure
A. Separation of HDL Cholesterol
   1. Reconstitute reagent according to instructions.
   2. Label tubes for appropriate controls and patients.
   3. Pipette 0.5 ml (500 ul) sample into respective tubes.
   4. Pipette 0.05 ml (50 ul) reagent into each tube and mix using vortex.
   5. Let tubes stand for five minutes at room temperature.
   6. Mix tubes with vortex and centrifuge at 1000-2000g for at least five minutes.

B. HDL Cholesterol Determination
   1. Label tubes “Blank”, “Standard”, “Controls”, “Patients”, etc.
   2. Pipette 1.0ml enzymatic cholesterol reagent into each tube.
   3. Pipette 0.05 ml (50 ul) standard or clear supernatants (from step #6 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
1. Automated instrumentation may be used for the “HDL Cholesterol Determination” procedure. Refer to appropriate instrument application instructions.
2. Hemolyzed specimens may be used if a sample blank is prepared to correct for hemolysis. In the “HDL Cholesterol Determination” procedure, add 0.05ml (50ul) to 1.0ml saline. Read against water at 500 nm, and subtract the absorbance reading from the test absorbance.

3. Certain samples will show incomplete sedimentation of the precipitate. For these samples, dilute the sample 1:1 with saline and repeat the precipitation step. The HDL Cholesterol result should be multiplied by two to compensate for the dilution.

Limitations
Icteric specimens should not be used and hemolyzed specimens require the use of a sample blank. See "Procedure Notes". Certain samples will show incomplete sedimentation of the precipitate. A 1:1 dilution with saline is necessary for the precipitation of these samples. See "Procedure Notes". Elevated levels of ascorbic acid inhibit the enzymatic cholesterol determination.

Calibration
Use an aqueous Cholesterol Standard (50 mg/dl) or an appropriate serum calibrator.

Calculations
$$\text{HDL Cholesterol (mg/dl)} = \frac{\text{Abs. Sample}}{\text{Abs. Std}} \times \text{Conc. of Std.} \times 1.1$$

Where 1.1 is the dilution factor from the HDL Separation procedure.

Sample calculation: If Abs. sample = .290, Abs. Std. = 0.250, and concentration of standard = 50 mg/dl then:

$$\frac{0.290}{0.250} \times 50 \times 1.1 = 64 \text{ mg/dl}$$

NOTE: To obtain values in S.I. units multiply mg/dl by 0.0259.

Quality Control
To monitor the reliability of results, control sera with known HDL cholesterol values should be run with patient samples.

Expected Values[19]
Males 27-78 mg/dl
Females 33-98 mg/dl

It is strongly recommended that each laboratory establish its own normal ranges. Information relating HDL Cholesterol value to risk of coronary heart disease is contained in references 12-17.

Performance
1. Comparison: A study was done between this reagent and another commercially available Mg/Dextran Sulfate precipitating reagent yielding a correlation coefficient of 0.999 with a linear regression equation of y=0.98x + 0.5. A total of 50 sera were assayed ranging in value from 5 to 129 mg/dl.
2. Precision:

Within Run			Run to Run		
Mean	**S.D.**	**C.V.%**	**Mean**	**S.D.**	**C.V.%**
32	0.5	1.6	33	1.7	3.9
63	0.8	1.3	73	2.3	3.8

3. Sensitivity: Following the procedure described using an enzymatic cholesterol reagent, 1 mg/dl HDL cholesterol will produce an absorbance of approximately 0.006.

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