HDL-Cholesterol Reagent

RAICHEM CE

CLINIQA®

Order No.	Description
R82051	5 × 5 mL, with Standard

INTENDED USE

The reagent is intended for the in vitro precipitation of very low density (VLDL) and low density (LDL) lipoprotein from serum or plasma. The clear supernatant remaining contains high density lipoprotein (HDL); this is used for the determination of HDL cholesterol.

TEST SUMMARY

The concentration of the cholesterol fraction in serum bound to high density lipoprotein has been shown to bear an inverse relationship to atherosclerotic vascular disease. Its determination is therefore important in identifying patients with high risk of developing coronary artery disease.[1-5] Numerous methods have been used to separate lipoprotein including ultracentrifugation, electrophoresis and precipitation. Procedures based on separation of VLDL and LDL lipoprotein with divalent cations and polyanions by precipitation have gained wide acceptance because they are relatively inexpensive and simple to use.[6,7,8] In the present method VLDL and LDL are quantitatively precipitated from serum by dextran sulfate and magnesium. After centrifugation, the HDL cholesterol fraction is determined in the clear supernatant by an enzymatic procedure. The magnesium-dextran sulfate procedure does not interfere with enzymatic methods of cholesterol assay.[9] This application for this method has not been tested or certified by the Cholesterol Reference Method Laboratory Network.

REAGENT COMPOSITION

When the reagent is dissolved as per directions the components have the following concentration:

Precipitating reagent

Reactive ingredients:

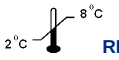
Dextran sulfate (Mol. Wt.	500,000)	10 g/L
Magnesium sulfate		1 mol/L

Non-reactive ingredients:

Buffers, stabilizers and fillers

REAGENT PREPARATION

Add to each vial of precipitating reagent 5 mL of distilled or deionized water. Cap the vial and mix vigorously several times. The reagent will go into solution in less than five minutes. The cholesterol standard provided does not require any additional treatment. Do not dilute.



REAGENT STORAGE AND STABILITY

The dry precipitating reagent in the original sealed container is stable until the expiration date on the vial when stored at 2–8 °C. The reconstituted precipitating reagent is stable for at least 60 days at room temperature. Avoid freezing. Store the cholesterol standard in the refrigerator at 2–8 °C.

If the solution of the reconstituted precipitating reagent is not clear or there is evidence of a precipitate, discard it.

PRECAUTIONS

Harmful by inhalation, in contact with skin and if swallowed. Irritating to eyes and skin.

Avoid contact with skin and eyes. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water for at least 10 minutes. If swallowed, seek medical advice immediately and show this container or label.

Good laboratory safety practices should be followed when handling any laboratory reagent. Refer to a recognized laboratory safety program for additional information. (See GP17-T, Clinical Laboratory Safety; Tentative Guideline (1994), National Committee on Clinical Laboratory Standards, Wayne, PA.)

Intended for in vitro diagnostic use only.

SPECIMEN COLLECTION, PREPARATION AND STORAGE

Collection of blood after overnight fasting is preferred; serum or EDTA plasma can be used for this assay. Hemolyzed samples are unsuitable for the determination.

HDL separation should be performed as soon as possible after collection of the specimen. If a delay is necessary, freeze the specimen. Frozen samples can be kept for several weeks at -15 °C and for at least two years at -50 °C. Refrigeration for longer than two weeks is not recommended.[9]

High turbidity in the samples indicates elevated levels of triglycerides. These samples may require additional treatment (dilution) to obtain a clean separation of lipoprotein.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Centrifuge.
2. Centrifuge tubes (13 x 100 mm test tubes can be used).
3. Pipettes to measure distilled or deionized water, samples and reagents.
4. Cholesterol Reagent.

   The following Cliniqa Cholesterol Reagents are recommended: Cholesterol Rapid Liquid Reagent, R85464 (2 x 120 mL); Cholesterol Enzymatic Reagent, Cat. No. R80015 (10 x 20 mL),or 80035 (10 x 50 mL).

   Spectrophotometer or colorimeter capable of measuring absorbance at 500 nm.
5. Matched cuvettes.

MATERIALS PROVIDED

HDL Cholesterol Reagent, consisting of 5 vials of precipitating reagent; 1 vial of 50 mg/dL cholesterol standard.

TEST PROCEDURE

A. Lipoprotein separation.

1. Pipette 1 mL each of water, controls and samples (serum or plasma) into appropriately labeled centrifuge tubes.
2. Add 0.1 mL of Cliniqa precipitating reagent.

(Note: If the sample volume is small, use 0.5 mL of sample and 0.05 mL of precipitating reagent)

3. Mix immediately for approximately 5 seconds in a vortex type mixer.
4. Let stand for 5 minutes at room temperature.
5. Centrifuge for 15 minutes at 2500 rpm.
6. The supernatant should be clear. Carefully remove the supernatant immediately without disturbing the precipitate and transfer to a clean, dry tube. Avoid lipid material that may occur floating on the surface. The clear supernatant can be stored up to two weeks in the refrigerator and up to three months at -20 °C.

(Note: Hazy or turbid supernatant may indicate incomplete precipitation and centrifugation, therefore the sample needs to be either further centrifuged, or diluted and re-centrifuged as described in step 7 below.

7. Turbidity in some samples indicates elevated triglycerides. Some of these samples may yield a turbid supernatant. Dilute these samples as follows: add to the mixture in the tube 1 mL of physiological saline (150 mmol/L sodium chloride in water) and 0.1 mL of precipitating reagent. Repeat steps 3, 4, 5 and 6. Multiply cholesterol results by 2.
8. The cholesterol standard is used without treatment with precipitating reagent.

B. Cholesterol assay. Use Cliniqa Cholesterol Enzymatic Reagent, Cat. Nos. R80015, R80035. Wavelength: 500 nm.

	Reagent	Blank	Standard	Sample
Water blank (Supernatant)	1 mL	50 µL	–	–
Standard 50 mg/dL	1 mL	–	50 µL	–
Sample (Supernatant)	1 mL	–	–	50 µL

Mix. Incubate for 10 minutes at 37 °C. Read the absorbance of the standard and samples with the instrument set at zero absorbance with the blank.

C. Perform a total cholesterol assay for each of the original serum or plasma samples using the procedure described in the Cholesterol Reagent direction insert.

CALIBRATION

This assay requires the use of a cholesterol standard. Use the standard provided with the reagent as directed. DO NOT dilute the cholesterol standard provided. The cholesterol standard is used without treatment with the precipitating reagent. Other commercially available standards and/or calibrators may be used.

QUALITY CONTROL

Serum controls are recommended to monitor the performance of manual and automated assay procedures, providing a continued screening of the instrument, reagents and techniques. Commercially available control

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material with established values for HDL cholesterol concentrations may be used. Assayed Control Serum Level 1 (Cat. No. R83082), Level 2 (Cat. No. R83083) and any commercially available Elevated Lipids Control are recommended for this purpose.

CALCULATIONS

$$\frac{A_s}{A_{std.}} \times \text{conc. of standard} \times \text{dilution factor} = \text{mg/dL HDL cholesterol.}$$

The dilution factor, 1.1, is used to compensate for the addition of the precipitating reagent to the serum.

For those samples which have been diluted as per Step 7 above, multiply the value obtained by 2.

Sample Calculation:

If the absorbance of the sample is 0.340 and that of the 50 mg/dL standard is 0.400 then:

$$\frac{0.340}{0.400} \times 50 \times 1.1 = 47 \text{ mg/dL HDL cholesterol.}$$

LIMITATIONS OF THE PROCEDURE

1. Supernatant samples with high concentrations of HDL cholesterol may indicate improper or delayed removal of the supernatant. Particular care must be taken not to disturb either the pellet of precipitated lipoprotein or any lipid material that may occur floating on the surface during pipetting of the sample. If this occurs, centrifuge the mixture again.
2. Turbidity in samples may indicate elevated triglycerides or non-fasting sample. Incomplete precipitation of LDL and VLDL may be occasionally observed in samples with elevated lipid levels. Process these samples as recommended at step 7 of Test Procedure.
3. Young et al.[10,11] published a list of drugs and other substances which have an effect on the level of cholesterol in serum or plasma or interfere with its determination.

REAGENT PERFORMANCE

1. Linearity: The concentration of HDL Cholesterol is rarely above 100 mg/dL. The Cliniqa Cholesterol Reagents listed above are linear to 500 mg/dL for total cholesterol.
2. Correlation: Employing as a reference a commercial reagent (Beckman), based on similar composition, in 98 samples ranging in HDL Cholesterol values between 13 and 89 mg/dL, the correlation coefficient was 0.981 and the regression equation was y = 0.99x + 0.16.
3. Precision:

The following results were obtained for 3 serum pools assayed 10 times each over 3 different days:

Within-Run:			
Mean (mg/dL)	28.8	48.9	69.2
SD (mg/dL)	0.76	1.76	1.08
CV (%)	2.6	3.6	1.6
Total:			
Mean (mg/dL)	28.8	48.9	69.2
SD (mg/dL)	0.75	1.74	1.04
CV (%)	2.6	3.6	1.5

REFERENCE RANGE

HDL-Cholesterol values vary considerably according to age and sex. The National Cholesterol Education Program (NCEP) guidelines are as follows.[12]
< 40 mg/dL (1.03 mmol/L): low HDL-Cholesterol (major risk factor for CHD)
≥ 60 mg/dL (1.55 mmol/L): high HDL-Cholesterol ("negative" risk factor for CHD)

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IVD **For in vitro diagnostic use**

See package insert for proper use

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RE-ORDER INFORMATION
HDL-Cholesterol Reagent

Catalog No.

REF R82051

Made in the USA

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