HDL-CHOLESTEROL CE

CONTENTS			
REF	1133010	HDL-Cholesterol	2 x 40 mL
For *in vitro* diagnostic use only			

HDL-CHOLESTEROL

DIFFERENTIAL PRECIPITATION
Enzymatic colorimetric test
ENDPOINT

PRINCIPLE

This technique[1] uses a separation method based on the selective precipitation of apoliprotein B-containing lipoproteins (VLDL, LDL and (a)Lpa) by phosphotungstic acid/$MgCl_2$, sedimentation of the precipitant by centrifugation, and subsequent enzymatic analysis of high density lipoproteins (HDL) as residual cholesterol remaining in the clear supernatant.

REAGENT COMPOSITION

R1 **Precipitating reagent**. Phosphotungstic acid 0.63 mmol/L, magnesium chloride 25 mmol/L. Stabilizers.

CAL **HDL-Cholesterol standard**. Cholesterol 50 mg/dL (0.38 mmol/L). Organic matrix based primary standard.

Cholesterol MR. Optative. Ref: 1118005, 1118010, 1118015.

STORAGE AND STABILITY

Store at 2-8ºC.
The reagents are stable until the expiry date stated on the label.

REAGENT PREPARATION

The Reagents and Standard are ready-to-use.

SAMPLES

Serum or EDTA plasma free of hemolysis obtained by the patient after an overnight fast. Remove from cells within 3 hours of venipuncture. Samples may be kept at 4-8ºC for 2 weeks, and at –20ºC for 3 months with no alteration of HDL cholesterol.
The supernate containing the HDL fraction is conveniently prepared on the day of sample collection and may be analysed after 2 weeks at 4-8ºC or 3 months at –20ºC in a non-selfdefrosting freezer.[2]

INTERFERENCES

- Hemoglobin (>200 mg/dL) and bilirubin (>10 mg/dL) do not interfere with the test.[3]
- Turbidity in samples may indicate elevated triglycerides or non-fasting specimens.

MATERIALS REQUIRED

I. *Precipitation*

- Dilutor and pipettes.
- Centrifuge tubes (13 x 100 m/m).
- Vortex mixer.
- Desktop centrifuge.

II . *Colorimetry*

- Kit for measuring Total Cholesterol.
- Constant temperature incubator set at 37ºC.
- Photometer or colorimeter capable of measuring absorbance at 550 ± 10 nm.

PROCEDURE

I. *Precipitation*

1. Bring reagents and samples to room temperature.
2. Pipette into labelled centrifuge tubes:

Sample or Standard	0.2 mL
Precipitating reagent	0.4 mL

Ratio $\frac{\text{Sample}}{\text{Reagent}} = \frac{1}{2}$

Dil. factor = 3

3. Vortex and allow to stand for 10 minutes at room temperature.
4. Centrifuge for 10 minutes at 4000 r.p.m., or 2 minutes at 12000 r.p.m.
5. Separate off the clear supernatant within 2 hours.

In case of turbid supernatants caused by elevated triglycerides (>350 g/dL) the sample should be diluted 1:2 with saline and steps 2,3,4 and 5 repeated. Multiply the result of the colorimetry by 2.

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II. *Colorimetry*

1. Bring the Cholesterol MR Monoreagent and the cholesterol standard (50 mg/dL) of the kit to room temperature
2. Pipette into labelled tubes:

TUBES	Blank	Sample Supernat	Standard Supernat
Monoreagent	1.0 mL	1.0 mL	1.0 mL
Supernat	–	50 µL	–
Standard	–	–	50 µL

3. Mix and let stand the tubes for 10 minutes at room temperature or 5 minutes at 37ºC.
4. Read the absorbance (A) of the supernatant and the standard at 550 nm against the reagent blank.

The color is stable for at least 30 minutes protected from light.

CALCULATIONS

$$\frac{A_{Supernatant}}{A_{Standard}} \times C_{Standard} = \text{mg/dL HDL-Cholesterol}$$

If results are to be expressed as SI units apply:
mg/dL x 0.0259 = mmol/L

REFERENCE VALUES[4]

Clinical values of HDL-Cholesterol used to classify risk groups.

Cholesterol from lipoproteins of high density		RISK
Men	> 55 mg/dL (> 1.42 mmol/L)	Low
	35-55 mg/dL (0.90-1.42 mmol/L)	Moderate
	< 40 mg/dL (< 1.04 mmol/L)	High
Women	> 65 mg/dL (> 1.68 mmol/L)	Low
	45-65 mg/dL (1.16-1.68 mmol/L)	Moderate
	< 45 mg/dL (< 1.16 mmol/L)	High

CLINICAL SIGNIFICANCE

Low HDL-cholesterol is a strong independent predictor of coronary heart disease. In ATP III[4], low HDL cholesterol is defined categorically as a level < 40 mg/dL (1.04 mmol/L), a change from the level of < 35 mg/dL in ATPII (1993).

Low HDL cholesterol is used as a risk factor to estimate 10-year risk for coronary heart disease, having several causes: elevated triglycerides, overweigh and obesity, physical inactivity, and type 2 diabetes. Other causes are, cigarrete smoking, very high carbohydrate intakes (> 60% of calories), and certain drugs as anabolic steroids and progestional agents.

ANALYTICAL PERFORMANCE

- ***Linearity.*** Up to 275 mg/dL
- ***Precision***

mg/dL	Within-run			Between-run		
Mean	42.1	45.8	54.6	42.1	45.8	54.6
SD	0.23	0.23	0.2	0.27	0.28	0.31
CV%	0.54	0.5	0.34	0.64	0.61	0.52
N	10	10	10	10	10	10

Replicates: 10 for each level. Replicates: 10 for each level
Instrument: CECIL CE 2021 for 8 days.

- ***Sensitivity.*** Using a 1:3 sample/reagent at 550 nm, 10 mg of cholesterol will produce a net absorbance of approximately 0.037.
- ***Correlation.*** This assay (y) was compared with a similar commercial method (x). The results were:
  N = 25 r = 0.995 y = 0.985 + 2.6

REFERENCES

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B1133-1/0312
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