BIOLABO
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HDL-CHOLESTEROL (PTA)

Precipitant

For treatment of specimens before quantitative determination of the HDL-Cholesterol in human serum and plasma

REF 86516	1 x 125 mL	Precipitant
	1 x 5 mL	Standard 100 mg/dL
REF 86536	1 x 30 mL	Precipitant
	1 x 5 mL	Standard 100 mg/dL

TECHNICAL SUPPORT AND ORDERS
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CE

IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (1) (7)

The principal role of high density lipoproteins (HDL) in lipid metabolism is the uptake and transport of cholesterol from peripherical tissues to the liver through a process known as reverse cholesterol transport. Low HDL cholesterol levels are strongly associated with an increased risk of coronary heart disease and coronary artery disease. Hence, the determination of serum HDL-Cholesterol is a useful tool in identifying high-risk patients. Increased Total Cholesterol/HDL-Cholesterol ratio is significant of an increased risk of atherosclerosis.

PRINCIPLE (8)

This reagent is only for treatment of specimens before determination of HDL-Cholesterol with a reagent for total cholesterol.

Low density lipoproteins (LDL), very low density (VLDL) and chylomicrons from specimens are precipitated by phosphotungstic acid (PTA) and Magnesium chloride. HDL-Cholesterol obtained in supernatant after centrifugation is then measured with Total Cholesterol reagent (i. e.: CHOLESTEROL CHOD-PAP BIOLABO REF 80106).

REAGENTS COMPOSITION

Vial R1 **PRECIPITANT**

Phosphotungstic acid (PTA)	13.9	mmol/L
Magnesium chloride	570	mmol/L

Vial R2 **STANDARD**

Cholesterol 100 mg/dL (2.58 mmol/L)

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use.

- Verify the integrity of the contents before use.
- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with skin and eyes, thoroughly wash affected areas with plenty of water.
- Reagents contain sodium azide (concentration < 0.1%) which may react with copper and lead plumbing. Flush with plenty of water when disposing.
- For further information, Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

REAGENTS PREPARATION

Reagent is ready for use.

SPECIMEN COLLECTION AND HANDLING (6)

Specimens should be collected after 12 h–14 h fasting.

Plasma: collected on EDTA.

Centrifuge and remove plasma from blood cells as soon as possible (within 3 hours).

Serum: Centrifuge and remove serum from blood cells as soon as possible (within 3 hours).

Avoid oxalate, fluoride, citrate or heparin.

HDL-Cholesterol in specimen is stable for:

- 1 to 3 days at 2-8° C
- 1 month at - 20° C.

STABILITY AND STORAGE

Store at 2-8° C , well recap in the original vial and away from light.

- Standard (vial R2): Transfer requested quantity, close the vial and store at 2-8° C.
- Without contamination, stored and used as described in the insert, reagents are stable until expiry date stated on the label.
- Aggregates in Precipitant (vial R1) are not significant of alteration, they will be precipitated during centrifugation.

INTERFERENCES (3) (5) (6)

PTA/Mg^{2+} procedure is less sensitive to hyperlipemia than CDC heparin/Mn^{2+} procedure. The procedure is sensitive especially to reaction conditions. It may be affected by temperature, timing in supernatant-precipitate separation.

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. HDL LDL CK-MB Calibrator REF 95506
3. HDL LDL CK-MB control sera (human origin)
   REF 95516 HDL LDL CK-MB and lipids Level 1
   REF 95526 HDL LDL CK-MB and lipids Level 2
4. Reagent for determination of total cholesterol (i. e.: BIOLABO CHOLESTEROL CHOD-PAP REF 80106)

Manufacturer | Use by | IVD In vitro diagnostic | Temperature limitation | REF Catalogue number | See insert | LOT Batch number | Store away from light | sufficient for | → dilute with

CALIBRATION (10)

- Use Standard (vial R2) provided in **this kit**, or equivalent.
  Do not treat Standard and multiply the result by 1.1.
- Or use HDL LDL CK-MB Calibrator REF 95506 (human serum traceable to SRM 3126a).
  Treat calibrator as sample and do not multiply the result by 1.1
- Sample volume: see table "Assay" § **MANUAL PROCEDURE**.

QUALITY CONTROL

- REF 95516 HDL LDL CK-MB and lipids Level 1
- REF 95526 HDL LDL CK-MB and lipids Level 2
- Or any assayed control sera of human origin referring to the same method (precipitation by PTA).
- External quality control program.

It is recommended to control in the following cases:

- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:

1. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control serum and repeat the test.
3. If control is still out of range, use a new vial of standard or equivalent and repeat the test.
4. If control is still out of range, calibrate with a new vial of reagent for determination of total cholesterol.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (9)

HDL-Cholesterol	mg/dL	[mmol/L]
Low level (Risk factor)	< 40	< 1.0
High level (Protective factor)	≥ 60	≥ 1.5

Each laboratory should establish its own normal ranges for the population that it serves.

PERFORMANCE CHARACTERISTICS

With reagent for Total Cholesterol (CHOD PAP) REF 80106:

Within run N = 10	*Medium level*	*High level*
Mean mg/dL	73	125
S.D. mg/dL	2.1	2.6
C.V. %	2.9	2.08

Between run N = 20	*Low level*	*High level*
Mean mg/dL	41	118
S.D. mg/dL	2.2	6.4
C.V. %	5.3	5.4

Detection limit: approximately 2 mg/dL.

Sensitivity: approx. 0.579 Abs for 100 mg/dL.

Comparison study with commercially available method:

y = 1.01x – 1 r = 0.9948

LINEARITY

When using BIOLABO Total Cholesterol (CHOD PAP) REF 80106:

The assay is linear up to 200 mg/dL (5.17 mmol/L). Above, dilute the supernatant with saline solution and re-assay taking into account the dilution factor. Linearity limit depends on specimen/reagent ratio.

MANUAL PROCEDURE

(*) Specimen, Calibrator and Controls Preparation:

Do not treat Standard (vial R2) enclosed in the kit.

Pipette in centrifuge tube	Macro-method	Micro-method
Specimen (*)	1 mL	0.5 mL
Precipitant	100 µL	50 µL

Mix vigorously, let stand for 10 minutes at room temperature.
Centrifuge 15 minutes at 3500-4000 RPM. (1500 g)

Then apply next procedure.

Assay:

With BIOLABO Total Cholesterol CHOD PAP REF 80106 or equivalent:

Let stand reagents and supernatants at room temperature.

Calibrate with standard enclosed in the kit or pre-treated seric calibrator

Pipette in well identified test tubes:	Blank	Standard	Assay
Reagent	1 mL	1 mL	1 mL
Demineralised water	25 µL		
Standard 100 mg/dL		25 µL	
Supernatant (*)			25 µL

Mix. Let stand for 5 minutes at 37° C or 10 minutes at room temperature.
Record absorbances at 500 nm (480-520) against reagent blank.
Colour is stable for 1 hour.

Notes:

- ✓ Supernatant: Specimen, controls REF 95516, REF 95526 and calibrator REF 95506 (human serum).
- ✓ Specific procedures for automated instruments are available upon request. Please contact BIOLABO technical support.
- ✓ If after centrifugation, supernatant remains cloudy, repeat the precipitation step with specimen diluted 1:2 in saline solution and multiply the result by 2.

CALCULATION

Calculate the result as follows:

Method n°1: with standard 100 mg/dL enclosed in the kit:

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{Standard concentration } \mathbf{x\ 1.1}$$

Standard remaining undiluted, 1.1 factor takes into account dilution of the specimen during the precipitation step.

Method n°2: with Calibrator REF 95506:

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (Calibrator)}} \times \text{Calibrator concentration}$$

Calibrator has been treated as sample, so do not multiply the result by 1.1.

REFERENCES

(1) *Badimon J. .J., Badimon L., Fuester V., Regression of atherosclerotic lesions by HDL plasma fraction in the Cholesterol-fed rabbit, Journal of clinical investigation, (1990), 85, p.1234-1241.*
(2) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 564-569*
(3) *YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p 3-308 à 3-317*
(4) *BURNSTEIN M and Al., Journal of Lipid Research,11, (1970), p.583-595*
(5) *LOPES-VIRELLA M. F .and Al., Clin. Chem.,(1977), 23/5, p.882-884*
(6) *NCEP-NIH Publication N°95-3044 (Sept. 1995) p.72-74.*
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(8) *TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 819-861.*
(9) *Recommandations de l'AFSSAPS sur la prise en charge thérapeutique du patient dyslipémique, p.9 (Mars 2005).*
(10) *SRM: Standard Reference Material ®*