BIOLABO
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MANUFACTURER:
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Non Esterified CHOLESTEROL

CHOD PAP Method

Reagent for quantitative determination of free Cholesterol in human serum or plasma

REF 88656	**R1**	2 x 100 mL	**R2**	2 x 100 mL	**R3**	1 x 5 mL
REF 99656	**R1**	6 x 500 mL	**R2**	6 x 500 mL	**R3**	1 x 5 mL

TECHNICAL SUPPORT AND ORDERS
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CE

IVD IN VITRO USE

CLINICAL SIGNIFICANCE (1) (5)

Cholesterol esterification is important because it allows to enhance lipids transport capability of lipoproteins from plasma and to prevent free cholesterol intracellular toxicity.
This kit for the determination of free cholesterol is for research use only.

PRINCIPLE (4)

Enzymatic method which reaction scheme is as follows:

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CO}} \text{Cholesten 4 one 3} + H_2O_2$$

$$2\ H_2O_2 + \text{Phenol} + \text{PAP} \xrightarrow{\text{POD}} \text{Quinoneimine (pink)} + 4\ H_2O$$

REAGENTS COMPOSITION

Vial R1 **BUFFER**

Phosphate buffer	100	mmol/L
Chloro-4-phenol	5	mmol/L
Sodium Cholate	2.3	mmol/L
Triton x 100	1.5	mmol/L
Preservative		

Vial R2 **ENZYMES**

Cholesterol oxydase (CO)	≥ 100	IU/L
Peroxydase (POD)	≥ 1200	IU/L
4 - Amino – antipyrine (PAP)	0.25	mmol/L
PEG 6000	167	µmol/L

Vial R3 **STANDARD**

Cholesterol 200 mg/dL (5,17 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 and seek medical advise.
- Reagents contain sodium azide (concentration < 0.1%) which may react with copper and lead plumbing. Flush with plenty of water when disposing.
- 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

Vial R2: use a non-sharp instrument to remove aluminium cap.
Add promptly the content of vial R2 (Enzymes), into vial R1 (Buffer).
Mix gently until complete dissolution (approximately 2 minutes).

STABILITY AND STORAGE

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

- Unopened, reagents are stable until expiry date stated on the label of the kit when stored and used as described in the insert.
- Standard (vial R3): Transfer the requested quantity, well recap and store at 2-8°C
- Once reconstituted, working reagent is stable for 2 years when free from contamination.
- Discard any reagent if cloudy or if reagent blank at 500 nm > 0.400.
- Don't use working reagent after expiry date stated on the label of the Kit.

SPECIMEN COLLECTION AND HANDLING (2)

Serum or plasma (Heparin or EDTA).
Do not use oxalate, fluoride or citrate. Collect on fasting patient ≥ 12 h is recommended. Patient should be sitting for 5 min before blood draw ; avoid prolonged tourniquet use.

Cholesterol is stable in the specimen for:

- 4-7 days at 2-8°C
- Avoid repeated freezing and thawing.

INTERFERENCES (3)

Interferences studies have led to following results:

- Ascorbic acid (AA): Negative interference above 2.5 mg/dL
- Haemoglobin (Hb): Positive interference above 400 µmol/L
- Glucose: No interference up to 1000 mg/dL
- Turbidity: Positive interference above 0.100 abs at 600nm (opalescent-milky serum)
- Bilirubin: No data yet available

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

MATERIALS REQUIRED BUT NOT PROVIDED

1. Basic medical analysis laboratory equipment.
2. Normal and pathological control sera

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

Made in France
Latest revision: www.biolabo.fr
Revision: 29/07/2011

CALIBRATION (6)

- Standard enclosed in the kit (vial R3) traceable to SRM 909b
- Or any calibrator traceable to reference method or material.

The calibration frequency depends on proper instrument functions and on the preservation of the reagent.

It is recommended to calibrate in the following cases:
1. When changing vial of reagent.
2. After maintenance operations on the instrument.
3. When control values are out of range, even after using a new vial of fresh serum.

QUALITY CONTROL

- REF 95516 Control serum HDL LDL CK-MB Lipids Level 1
  REF 95526 Control serum HDL LDL CK-MB Lipids Level 2
- Or any assayed control sera referring to the same method.
- 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 calibrator or a fresh calibrator and repeat the test.
4. If control is still out of range, calibrate with a new vial of reagent.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (3)

No data yet available. For research use only

PERFORMANCES

Within run n = 20	*Low level*	*Medium level*	*High level*
Mean mg/dL	23	58	200
S.D. mg/dL	0,4	1	2
C.V. %	1,72	1,96	0,99

Between run n = 20	*Low level*	*Medium level*	*High level*
Mean mg/dL	23	56	200
S.D.mg/dL	1	2	6
C.V. %	4.8	4.1	2.8

Detection limit: approximately 4 mg/dL at 37°C.

Sensitivity for 100 mg/dL: approximately 235 mAbs at 37°C.

Comparison study with commercially available reagent: Not yet available

LINEARITY

The reaction is linear up to 400 mg/dL.

Above, dilute the specimen (1 + 4) with saline solution 9 g/L and reassay taking into account the dilution factor to calculate the result. Linearity limit depends on specimen/reagent ratio.

MANUAL PROCEDURE

Let stand reagent and specimens at room temperature.

Pipette into well identified test tubes:	**Blank**	**Standard**	**Assay**
Reagent	1 mL	1 mL	1 mL
Demineralised water	10 µL		
Standard		10 µL	
Specimen			10 µL

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

Note: Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.

CALCULATION

Calculate the result as follows:

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

REFERENCES

(1) TIETZ N.W. Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders (1999) p. 826-835.
(2) Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p. 244-249.
(3) YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p. 3-143 to 3-164
(4) Allain C. C. et al., Clin. Chem. (1974), 20/4, p.470-475
(5) Allan C., Deacon et Peter J. G. Dawson, Clin. Chem. (1979) 25/6, p.976-984
(6) SRM: Standard Reference Material ®