BIOLABO
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CHOLESTEROL

CHOD PAP Method

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

REF 80106	**R1**	2 x 100 mL	**R2**	2 x 100 mL	**R3**	1 x 5 mL
REF 87356	**R1**	10 x 100 mL	**R2**	10 x 100 mL	**R3**	1 x 5 mL
REF 87656	**R1**	6 x 500 mL	**R2**	6 x 500 mL	**R3**	1 x 10 mL

TECHNICAL SUPPORT AND ORDERS
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CE

IVD IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (1) (2)

Total cholesterol assay, associated to assays of others lipids in serum is used in the diagnosis of hyperlipidemia. Increased levels are also seen in hepatic and thyroïd disorders.
Total cholesterol assay associated to triglycerides, HDL-Cholesterol and LDL-Cholesterol determination is useful in the prediction of coronary heart diseases.
So this assay is used in the diagnosis and treatment of atherosclerotic diseases. Hypercholesterolemia can also be observed in certain cases of diabetus. Secondary disorders that elevate cholesterol levels, should be ruled prior to initiating therapy with cholesterol-lowering drugs.

PRINCIPLE (4)

Enzymatic method described by Allain and al., which reaction scheme is as follows:

Cholesterol esters $\xrightarrow{\text{CE}}$ Cholesterol + free fatty acids

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

2 H_2O_2 + Phenol + PAP $\xrightarrow{\text{POD}}$ 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
Cholesterol esterase (CE)	≥ 170	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

Add promptly the content of vial R2 (Enzymes), into vial R1 (Buffer).

Mix gently until complete dissolution (approximately 2 minutes).

Vial R2: If appropriate, use a non-sharp instrument to remove aluminium cap.

STABILITY AND STORAGE

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

- Standard (vial R3): Transfer the requested quantity, recap and store at 2-8°C.
- Reagents are stable until expiry date stated on the label of the kit when stored and used as described in the insert.
- Working reagent is stable at least for 2 years.
- 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. Separate serum from cells within 2 hours.

Cholesterol is stable in the specimen for:

- 5-7 days at 2-8°C
- 3 months at –20°C
- Many years at –70°C.
- Avoid repeated freezing and thawing

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

Made in France
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INTERFERENCES (2) (3) (5)

Ascorbic acid: Negative interference above 5 mg/dL.
Haemoglobin: Positive interference above 33 mg/dL.
Bilirubin: Negative interference above 8.6 mg/dL.
Serum blank may increase this interference.
Lipemia: Low interference limited by CE lipase activity.

Enzymatic methods increase analytic specificity . CO also reacts with 3β-hydroxycholesterols (insignificant quantity in human serum – i.e. DHEA, pregnenolone).

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

MATERIALS REQUIRED BUT NOT PROVIDED

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

CALIBRATION (6)

- Kit standard (vial R3) or BIOLABO Multicalibrator REF 95015
- 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

- BIOLABO EXATROL-N Level I REF 95010.
- BIOLABO EXATROL-P Level II REF 95011.
- Other 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 (2)

Values for adults, estimated in term of risk for atherosclerotic diseases:

Total cholesterol	mg/dL	[mmol/L]
Recommended values	< 200	[< 5.18]
Low risk	200-239	[5.18-6.19]
High risk	≥ 240	[≥ 6.22]

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

PERFORMANCE CHARACTERISTICS (4)

Within run N = 30	*Normal level*	*High level*
Mean mg/dL	149	217
S.D. mg/dL	1.25	1.89
C.V. %	0.84	0.87

Between run: N = 33	*Normal level*	*High level*
Mean mg/dL	125	261
S.D. mg/dL	1.04	2.06
C.V. %	0.83	0.79

Detection limit: approximately 1 mg/dL
Sensitivity for 100 mg/dL: 0.235 ± 0.035
Comparison study with commercially available reagent:
y = 0.957 x + 6.4 r = 0.9904

LINEARITY

The reaction is linear up to at least 500 mg/dL (12.9 mmol/L).). Above, dilute the specimen with saline solution and reassay taking into account dilution factor. 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 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.

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 ®