Clinical Chemistry

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ABX Pentra LDL Direct CP

REF A11A01638
REAGENT 1 28 mL
REAGENT 2 10 mL

IVD CE

HORIBA ABX SAS
Parc Euromédecine
Rue du Caducée
BP 7290
34184 Montpellier Cedex 4
FRANCE

- Pentra C200

Diagnostic reagent for quantitative *in vitro* determination of Low Density Lipoprotein Cholesterol (LDL-C) in serum or plasma by colorimetry.

Application Release [a]

Serum, plasma: LDL

01.xx

Intended Use [a]

ABX Pentra LDL Direct CP reagent is intended for the quantitative *in vitro* diagnostic determination of Low Density Lipoprotein Cholesterol (LDL-C) in human serum and plasma based on an enzymatic colorimetric assay. Lipoprotein measurements are used in the diagnosis and treatment of lipid disorders, atherosclerosis, and various liver and renal diseases.

Clinical Interest

Plasma lipoproteins are spherical particles containing varying amounts of cholesterol, triglycerides, phospholipids and proteins. The phospholipid, free cholesterol and protein constitute the outer surface of the lipoprotein particle, while the inner core contains mostly esterified cholesterol and triglyceride. These particles serve to solubilize and transport cholesterol and triglyceride in the bloodstream.
The relative proportions of protein and lipid determine the density of these lipoproteins and provide a basis on which to begin their classification (1). These classes are: chylomicrons, very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Numerous clinical studies have shown that the different lipoprotein classes have very distinct and various effects on coronary heart disease risk (2, 3, 4). The studies all point to LDL cholesterol as the key factor in the pathogenesis of atherosclerosis and coronary artery disease (CAD) (2, 3, 4, 5, 6, 7, 8), while HDL cholesterol has been observed to have a protective effect. Even within the normal range of total cholesterol concentrations, an increase in LDL cholesterol can occur with an associated increased risk for CAD (4).

Method

ABX Pentra LDL Direct CP assay is an homogeneous method for directly measuring LDL-C levels in serum or plasma, without the need for any off-line pretreatment or centrifugation steps.
The method is in a two reagent format and depends on the properties of a unique detergent. This detergent (Reagent 1) solubilizes only the non LDL lipoprotein particles. The cholesterol released is consumed by cholesterol esterase and cholesterol oxidase in a non color forming reaction. A second detergent (Reagent 2) solubilizes the remaining LDL particles and a chromogenic coupler allows for color formation. The enzyme reaction with LDL-C in the presence of the coupler produces color that is proportional to the amount of LDL cholesterol present in the sample.

Reagents

ABX Pentra LDL Direct CP is ready-to-use.

Reagent 1:	
MES buffer pH 6.3	
Detergent 1	< 1.0%
Cholesterol Esterase	< 1500 U/L
Cholesterol Oxidase	< 1500 U/L

[a]Modification: chapter added.

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Reagent 1:	
Peroxidase	< 1300 ppg U/L
4-aminoantipyrine	< 0.1%
Ascorbic Acid Oxidase	< 3000 U/L
Preservative	
Reagent 2:	
MES buffer pH 6.3	
Detergent 2	< 1.0%
N,N-bis(4-sulfobutyl)-toluidine, disodium (DsBmT)	< 1.0 mM
Preservative	

ABX Pentra LDL Direct CP should be used according to this notice. The manufacturer cannot guarantee its performance if used otherwise.

Handling

1. Remove both caps of the cassette.
2. If present, remove foam by using a plastic pipette.
3. Place the cassette into the refrigerated Pentra C200 reagent compartment.

Calibrator

For calibration, use:
ABX Pentra LDL Cal (A11A01678) (not included)
2 x 1 mL (lyophilisate)

Control [b]

For internal quality control, use:

- **ABX Pentra N Control / ABX Pentra N MultiControl** (A11A01653 / 1300054414) (not included)
  10 x 5 mL (lyophilisate)
- **ABX Pentra P Control / ABX Pentra P MultiControl** (A11A01654 / 1300054415) (not included)
  10 x 5 mL (lyophilisate)

Each control should be assayed daily and/or after a calibration.
The frequency of controls and the confidence intervals should correspond to laboratory guidelines and country-specific directives. You should follow federal, state and local guidelines for testing quality control materials. The results must be within the range of the defined confidence limits. Each laboratory should establish a procedure to follow if the results exceed these confidence limits.

Materials Required but not Provided [b]

- Automated clinical chemistry analyzer: Pentra C200
- Calibrator: **ABX Pentra LDL Cal** (A11A01678)
- Controls:
  ABX Pentra N Control / ABX Pentra N MultiControl (A11A01653 / 1300054414)
  ABX Pentra P Control / ABX Pentra P MultiControl (A11A01654 / 1300054415)
- Standard laboratory equipment.

Specimen

- Serum.
- Plasma in lithium heparin.

Anticoagulants other than those listed have not been tested by HORIBA Medical and are therefore not recommended for use with this assay.

These specimens should be drawn from the patient after 12 - 14h fast.

Stability (9)**:**

Serum sample must be stored in closed containers at 4°C. Freezing can lead to falsely low values.

- Serum: Collect whole blood by venipuncture and allow to clot. Centrifuge and remove the serum as soon as possible (within 3 hours) (9).
- Plasma: Centrifuge and remove the plasma as soon as possible after collection (within 3 hours) (9).

Nota: Anticoagulants containing citrate should not be used.

Reference Range (10)

Each laboratory should establish its own reference ranges. The values given here are used as guidelines only.

The following NCEP cutpoints for patient classification are used for the prevention and management of coronary heart disease.

[b]Modification: new control.

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LDL Cholesterol	Classification
< 130 mg/dL (< 3.36 mmol/L)	Desirable
130-159 mg/dL (3.36-4.11 mmol/L)	Borderline High Risk
160 mg/dL (4.14 mmol/L)	High Risk

Storage and Stability

Stability before opening:

Stable up to the expiry date on the label if stored at 2-8°C.

Stability after opening:

Refer to the paragraph "Performance on Pentra C200".

Do not freeze.

Waste Management

Please refer to local legal requirements.

General Precautions [c]

- This reagent is for professional *in vitro* diagnostic use only.
- For prescription use only.
- This reagent is classified as non-hazardous in compliance with regulation (EC) N°.1272/2008.
- **Reagent 1 (R1):**
  Warning: This reagent is obtained from substances of animal origin. Consequently, it should be treated as potentially infectious and handled with the appropriate cautions in accordance with good laboratory practices (11).
- Do not pipette by mouth.
- Do not replenish the reagents.
- Do not swallow. Avoid contact with skin and mucous membranes.
- Observe the standard laboratory precautions for use.
- The reagent cassettes are disposable and should be disposed of in accordance with the local legal requirements.
- Please refer to the SDS associated with the reagent.
- Do not use the product if there is visible evidence of biological, chemical or physical deterioration.
- It is the user's responsibility to verify that this document is applicable to the reagent used.

Performance on Pentra C200

Serum, plasma

The performance data listed below have been obtained on the Pentra C200 analyzer.
The assay has not been tested or certified to meet CRMLN laboratory criteria.

Number of tests: approximately 104 tests

On Board Reagent Stability

Once opened, the reagent cassette placed in the refrigerated Pentra C200 compartment is stable for 66 days.

Sample volume: 2 µL/test

Limit of Quantitation

The limit of quantitation is determined according to CLSI (NCCLS), EP17-A protocol (12) and equals 0.14 mmol/L (5.42 mg/dL).

Accuracy and Precision

Repeatability (within-run precision)

Repeatability according to the recommendations found in the Valtec protocol (13) with samples tested 20 times:

- 2 controls
- 3 specimens (low / medium / high levels)

	Mean value mmol/L	Mean value mg/dL	CV %
Control specimen 1	1.18	45.55	1.83
Control specimen 2	1.60	61.86	1.77
Specimen 1	2.66	102.81	1.47
Specimen 2	3.35	129.80	1.27
Specimen 3	4.76	184.25	1.20

Reproducibility (total precision)

Reproducibility according to the recommendations found in the CLSI (NCCLS), EP5-A2 protocol (14) with samples tested in duplicate for 20 days (2 series per day):

- 2 controls
- 3 specimens (low / medium / high levels)

[c]Modification: general precautions modification.

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	Mean value mmol/L	Mean value mg/dL	CV %
Control specimen 1	1.22	47.21	3.43
Control specimen 2	1.62	62.64	5.22
Specimen 1	2.69	103.92	4.49
Specimen 2	3.28	126.91	2.83
Specimen 3	4.79	185.48	3.64

Measuring Range

The assay confirmed a measuring range from 0.14 mmol/L (5.42 mg/dL) to 10 mmol/L (387 mg/dL).
The reagent linearity has been assessed up to 10 mmol/L (387 mg/dL) according to the recommendations found in the CLSI (NCCLS), EP6-A protocol (15).

Correlation

Patient samples: Serum
Number of patient samples: 93
Specimens are correlated with a commercial reagent taken as reference according to the recommendations found in the CLSI (NCCLS), EP9-A2 protocol (16).
Values ranged from 0.25 mmol/L (9.68 mg/dL) to 9.48 mmol/L (366.88 mg/dL).
The equation for the allometric line obtained using Passing-Bablock regression procedure (17) is:
Y = 1.03 X - 0.10 (mmol/L)
Y = 1.03 X - 4.19 (mg/dL)
with a correlation coefficient r^2 = 0.9861.

Interferences

Haemoglobin: No significant influence is observed up to 350 µmol/L (603 mg/dL).
Lipemia: No significant influence is observed up to an Intralipid® concentration (representative of lipemia) of 200.0 mg/dL.
Total Bilirubin: No significant influence is observed up to 500 µmol/L (29.3 mg/dL).
Direct Bilirubin: No significant influence is observed up to 250 µmol/L (14.6 mg/dL).
Other limitations are given by Young as a list of drugs and preanalytical variables known to affect this methodology (18, 19).

Calibration Stability

The reagent is calibrated on Day 0. The calibration stability is checked by testing 2 control specimens.
The calibration stability is 43 days.
Note: A recalibration is recommended when reagent lots change, and when quality control results fall outside the range established.

Conversion Factor

mmol/L x 0.387 = g/L
mmol/L x 38.7 = mg/dL

Reference

1. Centers for Disease Control/National Institutes of Health Manual, ''Biosafety in Microbiological and Biomedical Laboratories'', 1988. I have also seen this as: Richardson JH and Barkley WE. eds. Biosafety in Microbiological and Biomedical Laboratories, U.S. Dept. of Health and Human Services, Public Health Service, HHS Publication No. (CDC) 84-8395, Washington, DC (1984).
2. National Comittee for Clinical Laboratory Standards, Preparation and Testing of Reagent Water in the Clinical Laboratory - Third Edition; Approved Guideline NCCLS Document C3-A3 (1997).
3. Gotto AM. Lipoprotein metabolism and the etiology of hyperlipidemia, Hospital Practice (1988) **23** (Suppl. 1): 4-13.
4. Crouse JR, Parks JS, Schey HM, Kahl FR. Studies of low density lipoprotein molecular weight in human beings with coronary artery disease. J. Lipid Res. (1985) **26** (5): 566-574.
5. Badimon JJ, Badimon L, Fuester V. Regression of Atherosclerotic Lesions by High Density Lipoprotein Plasma Fraction in the Cholesterol-Fed Rabbit. Journal of Clinical Investigation (1990) **85**: 1234-1241.
6. Castelli WP, Doyle JT, Gordon T, Hames CG, Hjortland MC, Hulley SB, Kagan A, Zukel WJ. HDL Cholesterol and other lipids in coronary heart disease. Circulation (1977) **55**: 767-772.
7. Barr DP, Russ EM, Eder HA. Protein-lipid relationships in human plasma. Am. J. Med. (1951) **11**: 480.
8. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. Am. J. Med. (1977) **62**: 707-714.
9. Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: THBooks Verlagsgesellschaft (1998): 172.
10. Bachorik PS, Ross JW. National Cholesterol Education Program Recommendations for Measurement of Low-Density Lipoprotein Cholesterol: Executive Summary, Clin. Chem. (1995) **41** (10):1414-1420.
11. Council Directive (2000/54/EC). Official Journal of the European Communities. No. L262 from October 17, 2000: 21-45.
12. Protocols for determination of limits of detection and limits of quantitation. Approved Guideline, CLSI (NCCLS) document EP17-A (2004) **24** (34).

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13. Vassault A, Grafmeyer D, Naudin C et al. Protocole de validation de techniques (document B). Ann. Biol. Clin. (1986) **44**: 686-745.
14. Evaluation of Precision Performance of Quantitative Measurement Method. Approved Guideline, CLSI (NCCLS) document EP5-A2 (2004) **24** (25).
15. Evaluation of the Linearity of Quantitative Analytical Methods. Approved Guideline, CLSI (NCCLS) document EP6-A (2003) **23** (16).
16. Method Comparison and Bias Estimation Using Patient Samples. Approved Guideline, 2nd ed., CLSI (NCCLS) document EP9-A2 (2002) **22** (19).
17. Passing H, Bablock W. A new biometrical procedure for testing the equality of measurements from two different analytical methods. J. Clin. Chem. Clin. Biochem. (1983) **21**: 709-20.
18. Young DS. Effects of Drugs on Clinical Laboratory Tests. 4th Edition, Washington, DC, AACC Press (1997) **3**: 143-163.
19. Young DS. Effects of Preanalytical Variables on Clinical Laboratory Tests. 2nd Edition, Washington, DC, AACC Press (1997) **3**: 120-132.