Clinical Chemistry

2026/01/05
A93A01252GUS

ABX Pentra HDL Direct CP

REF A11A01636
REAGENT 1 62 mL
REAGENT 2 21 mL

IVD CE 2797

HORIBA ABX SAS
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FRANCE

- Pentra C200

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

Application Release

Serum, plasma: HDL

01.xx

Intended Use [a] [b] [c]

ABX Pentra HDL Direct CP reagent is intended for the quantitative *in vitro* diagnostic determination of High Density Lipoprotein Cholesterol (HDL-C) in human serum and plasma based on an enzymatic assay with accelerator selective detergent methodology.
Clinical laboratories use.
Lipoprotein measurements are used in the diagnosis and treatment of lipid disorders, atherosclerosis, and various liver and renal diseases.
Assessing physiologic and pathologic variations of High-Density Lipoprotein Cholesterol (HDL-C) concentration in human serum and plasma is useful for screening or follow-up of these 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). The classes are: chylomicron, 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 varied effects on coronary heart disease risk (2).
The principle role of HDL in lipid metabolism is the uptake and transport of cholesterol from peripheral tissues to the liver through a process known as reverse cholesterol transport (a proposed cardioprotective mechanism) (3). Low HDL-C levels are strongly associated with an increased risk of coronary heart disease and coronary artery disease (4, 5, 6, 7, 8, 9). Hence, the determination of serum HDL-C is a useful tool in identifying high-risk patients. The Adult Treatment Panel of the National Cholesterol Education Program (NCEP) recommends that in all adults 20 years of age and over, a fasting lipoprotein profile (total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride) should be obtained once every five years to screen for coronary heart disease risk (10).
The reference method for the quantitation of HDL-C combines ultracentrifugation and chemical precipitation to separate HDL from other lipoproteins, followed by cholesterol measurement by Abell-Kendall analysis (11). This method is too time consuming and labor intensive for use in routine analysis (12). The first routine methods widely utilized by laboratories involved selective precipitation and removal of LDL and VLDL, followed by the enzymatic measurement of HDL-C in the supernatant fraction (11). Since these methods require off-line pretreatment and separation steps the assay procedures cannot be fully automated. As a result, routine determination of HDL-C has suffered from long handling times and poor reproducibility.

[a]Modification: modification of Intended Use chapter.
[b]Modification: modification of CE mark.
[c]Modification: new leaflet form.

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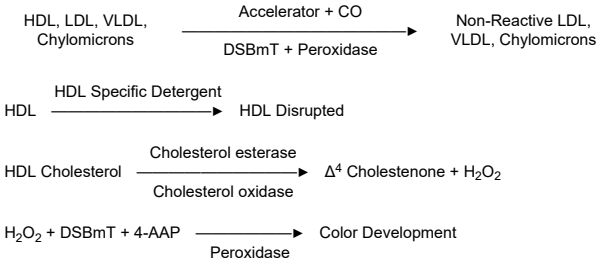
Method

ABX Pentra HDL Direct CP (Licensed under PCT/JP97/04442, PCT/JP00/03860) assay is a homogeneous method for directly measuring HDL-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, as illustrated. This method is based on accelerating the reaction of cholesterol oxidase (CO) with non-HDL unesterified cholesterol and dissolving HDL selectively using a specific detergent.
In the first reagent, non-HDL unesterified cholesterol is subject to an enzyme reaction and the peroxide generated is consumed by a peroxidase reaction with DSBmT yielding a colorless product.
The second reagent consists of a detergent capable of solubilizing HDL specifically, cholesterol esterase (CE) and chromagenic coupler to develop color for the quantitative determination of HDL-C. This may be referred to as the Accelerator Selective Detergent methodology.



(4-AAP = 4-Aminoantipyrine, CO = Cholesterol Oxidase, DSBmT = N,N-bis(4- sulphobutyl)-m-toluidine-disodium)

Reagents

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

Reagent 1 (R1):	
Good's buffer	
Cholesterol oxidase	< 1000 U/L
Peroxidase	< 1300 ppg U/L
N,N-bis(4-sulfobutyl)-m-toluidinedisodium (DSBmT)	< 1 mmol/L
Accelerator	< 1 mmol/L
Preservative	< 0.06%
Ascorbic acid oxidase	< 3000 U/L

Reagent 2 (R2):	
Good's buffer	
Cholesterol esterase	< 1500 U/L
4-aminoantipyrine (4-AAP)	< 1 mmol/L
Detergent	< 2%
Preservative	

ABX Pentra HDL 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 HDL Cal (A11A01647) (not included)
2 x 1 mL (lyophilisate)
The value of **ABX Pentra HDL Cal** is assigned by procedures traceable to the National Reference System for Cholesterol (NRS/CHOL). Calibration materials have concentrations around the medical decision level.

Control [d]

For internal quality control, use:

- **ABX Pentra N MultiControl** (1300054414) (not included)
  10 x 5 mL (lyophilisate)
- **ABX Pentra P MultiControl** (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

[d]Modification: control removed.

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limits. Each laboratory should establish a procedure to follow if the results exceed these confidence limits.

Materials Required but not Provided [d]

- Automated clinical chemistry analyzer: Pentra C200
- Calibrator: **ABX Pentra HDL Cal** (A11A01647)
- Controls:
  ABX Pentra N MultiControl (1300054414)
  ABX Pentra P MultiControl (1300054415)
- Standard laboratory equipment.

Specimen [e]

This device intended testing population is general population.

Specimen types

- Serum.
- Plasma in EDTA.
- Plasma in lithium heparin.

Anticoagulants other than those listed have not been tested by HORIBA and are therefore not recommended for use with this assay.
These specimens should be drawn from the patient after 12 - 14h fast.

Stability (11)

- At 4°C: 2 days
- At -20°C with vials that have leak- and evaporation-proof seals: 1 month
- At -70°C with vials that have leak- and evaporation-proof seals: 2 years

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

Nota: Anticoagulants containing citrate should not be used.

Reference Range (9, 13) [f]

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

Men: 0.77 - 1.81 mmol/L (30 - 70 mg/dL)
Women: 0.77 - 2.19 mmol/L (30 - 85 mg/dL)

According to the NCEP, HDL values greater than or equal to 1.033 mmol/L (40 mg/dL) are considered desirable, and values greater than or equal to 1.550 mmol/L (60 mg/dL) are considered to offer some protection against coronary heart disease. Values below 1.033 mmol/L (40 mg/dL) are considered to be a significant independent risk factor for coronary heart disease (9).

Clinical sensitivity and specificity, positive predictive value and negative predictive value are not commonly reported for this analyte. This is largely attributed to the fact that this analyte is not sole indicator for the intended purpose and patient treatment decision making. To arrive at a diagnosis and a course of treatment, results from others routine clinical chemistry tests should be used in conjunction with other diagnostic information and the attending health-care professional's evaluation of the patient's condition.

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 [g]

- This reagent is for professional *in vitro* diagnostic use only.
  For laboratory use.

[d]Modification: control removed.
[e]Modification: modification of "Specimen".
[f]Modification: information added.
[g]Modification: general precautions modification.

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- For prescription use only.
- This reagent is classified as non-hazardous in compliance with regulation (EC) N°.1272/2008.
- 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.
- Do not use the product if the recommended storage conditions, including temperature, are not followed.
- User must be trained by a HORIBA representative before attempting to operate the device.
- It is the user's responsibility to verify that this document is applicable to the reagent used.
- For technical assistance, you can call +33 (0)4 67 14 15 16.
- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the country in which the user and/or the patient is established.

Performance on Pentra C200

Lot to Lot Variability [h]

The recovery of samples (serum and plasma) done during QC release of three consecutive lots of reagent shows that the lot to lot variability is within specification: < 10%.

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 238 tests

On Board Reagent Stability

Once opened, the reagent cassette placed in the refrigerated Pentra C200 compartment is stable for 35 days.

Sample volume: 2 µL/test

Detection Limit [i]

The detection limit is determined according to CLSI (NCCLS), EP17-A2 protocol (14) and equals 0.01 mmol/L (0.51 mg/dL).

Limit of Quantitation

The limit of quantitation is determined according to CLSI (NCCLS), EP17-A2 protocol (14) and equals 0.07 mmol/L (2.71 mg/dL).

Accuracy and Precision

Repeatability (within-run precision)

Repeatability according to the recommendations found in the Valtec protocol (15) 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	0.81	31.29	0.73
Control specimen 2	1.61	62.33	1.37
Specimen 1	0.76	29.55	1.15
Specimen 2	1.45	56.25	1.80
Specimen 3	2.34	90.44	1.78

Reproducibility (total precision)

Reproducibility according to the recommendations found in the CLSI (NCCLS), EP5-A2 protocol (16) with samples tested in duplicate for 20 days (2 series per day):

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

	Mean value mmol/L	Mean value mg/dL	CV %
Control specimen 1	0.84	32.41	2.7
Control specimen 2	1.65	63.75	3.4
Specimen 1	0.79	30.62	3.4
Specimen 2	1.48	57.35	3.2
Specimen 3	2.43	94.06	3.5

[h]Modification: chapter added.
[i]Modification: data added.

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Measuring Range

The assay confirmed a measuring range from 0.07 mmol/L (2.71 mg/dL) to 4.50 mmol/L (174.15 mg/dL). The reagent linearity has been assessed up to 4.50 mmol/L (174.15 mg/dL) according to the recommendations found in the CLSI (NCCLS), EP06-Ed2 protocol (17).

Correlation [j]

Patient samples: Serum
Number of patient samples: 122
Specimens are correlated with a commercial reagent taken as reference according to the recommendations found in the CLSI (NCCLS), EP09c protocol (18).
Values ranged from 0.44 mmol/L (17.03 mg/dL) to 4.37 mmol/L (169.12 mg/dL).
The equation for the allometric line obtained using Passing-Bablok regression procedure (19) is:
Y = 1.059 X - 0.092 (mmol/L)
Y = 1.059 X - 3.574 (mg/dL)
with a correlation coefficient r^2 = 0.994.

Interferences

Haemoglobin:	No significant influence is observed up to 350 µmol/L (603 mg/dL).
Triglycerides:	No significant influence is observed up to a triglyceride concentration of 4.52 mmol/L (395.5 mg/dL).
Total Bilirubin:	No significant influence is observed up to 100 µmol/L (5.9 mg/dL).
Direct Bilirubin:	No significant influence is observed up to 500 µmol/L (29.3 mg/dL).

Other limitations are given by Young as a list of drugs and preanalytical variables known to affect this methodology (20, 21).

Calibration Stability

The reagent is calibrated on Day 0. The calibration stability is checked by testing 2 control specimens.
The calibration stability is 6 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

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[j]Modification: modification of correlation.

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