Clinical Chemistry

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REAGENT 90 mL

IVD CE Rx Only

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ABX Pentra Cholesterol CP

- Pentra C200

Diagnostic reagent for quantitative *in vitro* determination of Cholesterol in serum or plasma by colorimetry.

Application Release

Serum, plasma: CHOL

01.xx

Intended Use

ABX Pentra Cholesterol CP reagent is intended for the quantitative *in vitro* diagnostic determination of cholesterol in human serum and plasma based on an enzymatic photometric test (Trinder's reaction). Cholesterol measurements are used in the diagnosis and treatment of disorders involving excess cholesterol in the blood and lipid and lipoprotein metabolism disorders.

Clinical Interest (1, 2)

Cholesterol is a component of cell membranes and a precursor for steroid hormones and bile acids synthesized by body cells and absorbed with food (1). Cholesterol is transported in plasma via lipoproteins, namely complexes between lipids and apolipoproteins (1). There are four classes of lipoproteins: high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL) and chylomicrons. While LDL is involved in the cholesterol transport to the peripheral cells, HDL is responsible for the cholesterol uptake from the cells. The four different lipoprotein classes show distinct relationship to coronary atherosclerosis (1). LDL-cholesterol (LDL-C) contributes to atherosclerotic plaque formation within the arterial intima and is strongly associated with coronary heart disease (CHD) and related mortality. Even with total cholesterol within the normal range an increased concentration of LDL-C indicates high risk. HDL-C has a protective effect impeding plaque formation and shows an inverse relationship to CHD prevalence. In fact, low HDL-C values constitute an independent risk factor. The determination of the individual total cholesterol (TC) level is used for screening purposes while for a better risk assessment it is necessary to measure additionally HDL-C and LDL-C.
In the last few years several controlled clinical trials using diet, life style changes and / or different drugs (especially HMG CoA reductase inhibitors [statins]) have demonstrated that lowering total cholesterol and LDL-C levels reduce drastically CHD risk (2).

Certification

Traceability to the National Reference System for Cholesterol was established by performing a direct comparison with the cholesterol reference method using human specimens that cover the National Cholesterol Education Program (NCEP) medical decision points.
The ability to meet the NCEP's performance criteria for accuracy was demonstrated by using the **ABX Pentra Cholesterol CP** reagent according to the manufacturer's instructions on the Pentra C200 analyzer (calibrated using the value assigned to the **ABX Pentra Multical**, ref.A11A01652).

The results of the direct comparison and precision studies are available on www.horiba-abx.com/documentation.
The **ABX Pentra Cholesterol CP** reagent is for use in Clinical Laboratories.

Method (3, 4)

''CHOD-PAP'': enzymatic photometric test.
Determination of cholesterol after enzymatic hydrolysis and oxidation (3, 4). The colorimetric indicator is quinoneimine which is generated from 4-aminoantipyrine

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and phenol by hydrogen peroxide under the catalytic action of peroxidase (Trinder's reaction) (3).

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHO}} \text{Cholesterol-3-one} + H_2O_2$$

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneime} + 4H_2O$$

(CHE = Cholesterol Esterase, CHO = Cholesterol Oxydase, POD = Peroxidase)

Reagents [a]

ABX Pentra Cholesterol CP is ready-to-use.

Reagent:	
Good's buffer pH 6.7	50 mmol/L
Phenol	5 mmol/L
4-aminoantipyrine (4-AAP)	0.3 mmol/L
Cholesterol esterase (CHE)	≥ 200 U/L
Cholesterol oxidase (CHO)	≥ 50 U/L
Peroxidase (POD)	≥ 3 kU/L

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

Handling

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

Calibrator

For calibration, use:
ABX Pentra Multical (A11A01652) (not included)
10 x 3 mL (lyophilisate)

Control [b]

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 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 Multical** (A11A01652)
- Controls:
  ABX Pentra N MultiControl (1300054414)
  ABX Pentra P MultiControl (1300054415)
- Standard laboratory equipment.

Specimen (5, 6, 7) [c]

This device intended testing population is general population.

- Serum.
- Plasma in EDTA (not for use in the USA).
- 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.

Limitations (5, 6, 7)**:**

These specimens should be drawn from the patient after 12 - 14 h fast. The patient should sit quietly for about 5 minutes before the sample is drawn.

[a]Modification: § "Reagents": modification.
[b]Modification: control removed.
[c]Modification: modification of "Specimen".

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Biological variability can be reduced by drawing blood under standardized conditions as recommended by NCEP.
NCEP recommends that cholesterol measurements should not be made from plasma derived from fluoride, citrate or oxalate treated specimens.

Stability (5)**:**

Cholesterol levels in the sample have been reported to be stable for 5 - 7 days at 4°C or room temperature, 3 months at -20°C and many years at -70°C.

Reference Range (2, 6, 8) [d]

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

Cholesterol	Classification
≤ 200 mg/dL (≤ 5.17 mmol/L)	Desirable
200 - 239 mg/dL (5.17 - 6.18 mmol/L)	Borderline high risk
> 240 mg/dL (> 6.21 mmol/L)	High risk

At least two measurements of cholesterol on separate occasions should be made before any medical decision is made since a single point total cholesterol measurement may not represent a patient's usual cholesterol concentration, and cholesterol results around the decision points should be followed with a repeat measurement.

The European Task Force on Coronary Prevention recommends to lower TC concentration to less than 190 mg/dL (5.0 mmol/L) and LDL cholesterol to less than 115 mg/dL (3.0 mmol/L) (2).

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 [e]

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".

Note: It has to be mentioned, that the measurement is not influenced by occasionally occurring color changes, as long as the absorbance of the reagent is < 0.3 at 546-nm.

Waste Management

- Please refer to local legal requirements.
- This reagent contains less than 0.1% of sodium azide as a preservative. Sodium azide may react with lead and copper to form explosive metal azides.

General Precautions [f]

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

[d]Modification: information added.
[e]Modification: modification of storage and stability.
[f]Modification: general precautions modification.

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- 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 [g]

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: +/- 8%.

Serum, plasma

The performance data listed below have been obtained on the Pentra C200 analyzer.

Number of tests: approximately 309 tests

On Board Reagent Stability

Once opened, the reagent cassette placed in the refrigerated Pentra C200 compartment is stable for 88 days.

Sample volume: 3 µL/test

Detection Limit [h]

The detection limit is determined according to CLSI (NCCLS), EP17-A protocol (9) and equals 0.01 mmol/L (0.44mg/dL).

Limit of Quantitation [i]

The limit of quantitation is determined according to CLSI (NCCLS), EP17-A2 protocol (10) and equals 0.20 mmol/L (8 mg/dL).

Accuracy and Precision

Repeatability (within-run precision)

Repeatability according to the recommendations found in the Valtec protocol (11) 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	2.62	101	1.08
Control specimen 2	4.93	191	1.52
Specimen 1	2.98	115	2.52
Specimen 2	5.43	210	0.47
Specimen 3	7.63	295	0.83

Reproducibility (total precision)

Reproducibility according to the recommendations found in the CLSI (NCCLS), EP5-A2 protocol (12) 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	2.75	106.42	2.2
Control specimen 2	5.25	203.07	2.1
Specimen 1	2.99	115.85	3.4
Specimen 2	5.37	207.68	2.3
Specimen 3	7.62	294.88	2.9

Measuring Range [j]

The assay confirmed a measuring range from 0.20 mmol/L (8.0 mg/dL) to 15 mmol/L (580.5 mg/dL).
The reagent linearity has been assessed up to 15 mmol/L (580.5 mg/dL) according to the recommendations found in the CLSI (NCCLS), EP06-Ed2 protocol (13).

Correlation [k]

Patient samples: Serum
Number of patient samples: 127
Specimens are correlated with a commercial reagent taken as reference according to the recommendations found in the CLSI (NCCLS), EP09c protocol (14).
Values ranged from 1.53 mmol/L (59.21 mg/dL) to 13.92 mmol/L (538.70 mg/dL).
The equation for the allometric line obtained using Passing-Bablok regression procedure (15) is:
Y = 1.045 X - 0.2301 (mmol/L)

[g]Modification: chapter added.
[h]Modification: data added.
[i]Modification: modification of quantitation limit.
[j]Modification: modification of measuring range.
[k]Modification: modification of correlation.

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Y = 1.045 X – 8.905 (mg/dL)
with a correlation coefficient r^2 = 0.992.

Interferences [I]

Haemoglobin:	No significant influence is observed up to 250 µmol/L (431 mg/dL).
Triglycerides:	No significant influence is observed up to a triglyceride concentration of 6.76 mmol/L 591.5 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 75 µmol/L (4.4 mg/dL).
N-Acetylcysteine (NAC):	No significant influence is observed up to 550 mg/L (55 mg/dL). Patients treated with N-Acetylcysteine (NAC) for Paracetamol overdose may generate a false low result.
N-acetyl-p-benzoquinone imine (NAPQI):	No significant influence is observed up to 500 µmol/L (7.5 mg/dL).

Other limitations are given by Young as a list of drugs and preanalytical variables known to affect this methodology (16, 17).

Calibration Stability

The reagent is calibrated on Day 0. The calibration stability is checked by testing 2 control specimens.
The calibration stability is 47 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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14. Measurement Procedure Comparison and Bias Estimation Using Patient Samples. Approved Guideline, 3rd ed., CLSI (NCCLS) document EP09c (2018) **38** (12).
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16. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th Edition, Washington, DC, AACC Press (2000).

[I]Modification: modification of interferences.

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17. Young DS. Effects of Preanalytical Variables on Clinical Laboratory Tests. 2nd Edition, Washington, DC, AACC Press (1997) **3**: 120-132.