Clinical Chemistry

2023/09/25
A93A01303FEN

REF A11A01640

REAGENT 90 mL

IVD CE

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

ABX Pentra Triglycerides CP

- **Pentra C400**

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

Application Release

Serum, plasma: Trigly

1.xx

Intended Use [a]

ABX Pentra Triglycerides CP reagent is intended for the quantitative *in vitro* diagnostic determination of triglycerides in human serum and plasma based on an enzymatic colorimetric assay. Measurements obtained by this device are used in the diagnosis and treatment of patients with diabetes mellitus, nephrosis, liver obstruction, other diseases involving lipid metabolism, or various endocrine disorders.

Clinical Interest (1, 2)

Triglycerides constitute 95% of fat stocked in tissues and their main role is to provide energy to cells. They are synthetised on one hand in the intestine from fat brought by food and on the other hand in liver from ingested saccharides, and are then transported in the blood by chylomycrons and very low density lipoproteins (VLDL).
High levels of triglycerides are associated with important risks of atherosclerosis. They may be caused by diseases such as different lipid metabolism troubles (hyperlipoproteinemia, deficit in lipase activity, deficit in apolipoprotein CII), but also by diabetes, renal or endocrinal troubles.

Method (3)

Enzymatic determination of triglycerides according to the following reactions:

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipoprotein lipase}} \text{Glycerol} + \text{fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerokinase}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-Phosphate} + O_2 \xrightarrow{\text{Glycerol-3-phosphate Oxidase}} H_2O_2 + \text{DHAP}$$

$$2H_2O_2 + \text{4-AAP} + \text{p-Chlorophenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4H_2O$$

(DHAP = Dihydroxyacetone phosphate, 4-AAP = 4-aminoantipyrine)

Reagents [b]

ABX Pentra Triglycerides CP is ready-to-use.

Reagent:	
Good's buffer pH 7.00	
4-Chlorophenol	2.7 mmol/L
ATP	3.15 mmol/L
4-aminoantipyrine (4-AAP)	0.31 mmol/L
Lipoprotein lipase	≥ 2000 U/L
Glycerokinase	≥ 500 U/L
Glycerol-3-phosphate-oxidase	≥ 4000 U/L
Peroxidase	≥ 500 U/L
Sodium azide	< 0.1%

[a]Modification: new leaflet form.
[b]Modification: § "Reagents": modification.

ABX Pentra Triglycerides CP

Also contains magnesium salt, FAD and detergents for optimal performance.

ABX Pentra Triglycerides 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 [c]

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 [c]

- Automated clinical chemistry analyzer: Pentra C400
- Calibrator: **ABX Pentra Multical** (A11A01652)
- Controls:
  ABX Pentra N MultiControl (1300054414)
  ABX Pentra P MultiControl (1300054415)
- Cleaning solutions:
  ABX Pentra Clean-Chem CP (A11A01755), 30 mL **or**
  ABX Pentra Clean-Chem 99 CP (A11A01789), 4 x 99 mL
- Standard laboratory equipment.

Specimen (4) [d]

This device intended testing population is general population.

Specimen types

- 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 (4)

No significant change of triglycerides concentration after storage for 4 days at 4°C.

Reference Range (2) [e]

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

In a study conducted within the NCEP (National Cholesterol Education Program, launched by the US Ministry of Health), the triglycerides values in serum have been classified according to the risk of developing cardiovascular diseases:

Normal:	< 150 mg/dL
Low risk:	150 - 200 mg/dL
High:	200 - 500 mg/dL
Extremely high:	> 500 mg/dL

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

[c]Modification: control removed.
[d]Modification: modification of "Specimen".
[e]Modification: information added.

ABX Pentra Triglycerides CP

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 C400".

Note: the reagents' colour may change to brown in the course of time, but this does not affect the reagent performance.

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.
- **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 (5).
- 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.
- 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 C400

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 are representative of performance on HORIBA Medical Systems.

Number of tests: 295 tests
If the number of tests requested is low and the Pentra C400 user intends to utilise the cassette to the maximum on board stability, it is the recommendation of HORIBA Medical, to utilise the consumable part XEC083 (Kit membrane) to achieve the number of tests stated in this notice.

On Board Reagent Stability

Once opened, the reagent cassette placed in the refrigerated Pentra C400 compartment is stable for 48 days.

Sample volume: 3 µL/test

Detection Limit [h]

The detection limit is determined according to CLSI (NCCLS), EP17-A2 protocol (6) and equals 0.13 mmol/L (11.38 mg/dL).

---

[f]Modification: general precautions modification.
[g]Modification: chapter added.
[h]Modification: modification of detection limit.

ABX Pentra Triglycerides CP

Limit of Quantitation [i]

The limit of quantitation is determined according to CLSI (NCCLS), EP17-A2 protocol (6) and equals 0.14 mmol/L (12.25 mg/dL).

Accuracy and Precision [j]

Repeatability (within-run precision)

Repeatability according to the recommendations found in the Valtec protocol (7) 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	1.44	126.2	2.52
Control specimen 2	2.44	213.6	0.82
Specimen 1	0.68	59.7	2.83
Specimen 2	1.24	108.4	1.84
Specimen 3	2.65	231.9	1.00

Reproducibility (total precision)

Reproducibility according to the recommendations found in the CLSI (NCCLS), EP5-A2 protocol (8) with samples tested in duplicate for 20 days (2 series per day):

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

	Mean value mmol/L	Mean value mg/dL	CV %
Control specimen 1	1.18	103.01	3.5
Control specimen 2	2.18	190.94	2.7
Specimen 1	1.41	123.08	2.8
Specimen 2	2.75	240.58	2.7

Measuring Range [k]

The assay confirmed a measuring range from 0.14 mmol/L (12.25 mg/dL) to 13 mmol/L (1137 mg/dL).
The measuring range is extended up to 52 mmol/L (4550 mg/dL) with the automatic post-dilution.

The reagent linearity has been assessed up to 13 mmol/L (1137 mg/dL) according to the recommendations found in the CLSI (NCCLS), EP06-Ed2 protocol (9).

Correlation [l]

Patient samples: Serum
Number of patient samples: 121
Specimens are correlated with a commercial reagent taken as reference according to the recommendations found in the CLSI (NCCLS), EP09c protocol (10).
Values ranged from 0.17 mmol/L (14.88 mg/dL) to 12.97 mmol/L (1134.87 mg/dL).
The equation for the allometric line obtained using Passing-Bablok regression procedure (11) is:
Y = 0.9856 x + 0.00174 (mmol/L)
Y = 0.9856 x + 0.1524 (mg/dL)
with a correlation coefficient r^2 = 0.998.

Interferences [m]

Haemoglobin:	No significant influence is observed up to 290 µmol/L (500 mg/dL).
Total Bilirubin:	No significant influence is observed up to 384.6 µmol/L (22.5 mg/dL).
Direct Bilirubin:	No significant influence is observed up to 385 µmol/L (22.5 mg/dL).
N-Acetylcysteine (NAC):	No significant influence is observed up to 1686 µmol/L (28 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 250 µmol/L (3.7 mg/dL).
Etamsylate:	No significant influence is observed up to 114 µmol/L (3.0 mg/dL).

The presence of N-Acetylbenzoquinoneimine (NAPQI) in serum/plasma can cause false results.

Other limitations are given by Young as a list of drugs and preanalytical variables known to affect this methodology (12, 13).

[i]Modification: data added.
[j]Modification: modification of accuracy and precision.
[k]Modification: modification of measuring range.
[l]Modification: modification of correlation.
[m]Modification: modification of interferences.

ABX Pentra Triglycerides CP

Calibration Stability

The reagent is calibrated on Day 0. The calibration stability is checked by testing 2 control specimens.
The calibration stability is 14 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 0.875 = g/L
mmol/L x 87.5 = mg/dL

Reference

1. Naito HK, Coronary Artery Disease and Disorders of Lipid Metabolism. Clinical Chemistry: Theory, Analysis, Correlation, 4ème Ed., Kaplan LA, Pesce AJ, Kazmierczak SC. (Mosby, Inc. eds. St Louis USA), (2003): 603.
2. Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP). JAMA, (2001) **285**: 2486.
3. Fossati P, Prencipe L, Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem. (1982) **28**: 2077.
4. Thomas L. Clinical Laboratory Diagnostics. 1st Ed. Frankfurt: TH-Books Verlagsgesellschaft, (1998): 169-170.
5. Council Directive (2000/54/EC). Official Journal of the European Communities. No. L262 from October 17, 2000: 21-45.
6. Evaluation of detection capability for clinical laboratory measurement procedures. Approved Guideline, 2nd ed., CLSI (NCCLS) document EP17-A2 (2012) **32** (8).
7. Vassault A, Grafmeyer D, Naudin C et al. Protocole de validation de techniques (document B). Ann. Biol. Clin. (1986) **44**: 686-745.
8. Evaluation of Precision Performance of Quantitative Measurement Method. Approved Guideline, CLSI (NCCLS) document EP5-A2 (2004) **24** (25).
9. Evaluation of Linearity of Quantitative Measurement Procedures. 2nd Edition, CLSI (NCCLS) guideline EP06-Ed2 (2020) **40** (16).
10. Measurement Procedure Comparison and Bias Estimation Using Patient Samples. Approved Guideline, 3rd ed., CLSI (NCCLS) document EP09c (2018) **38** (12).
11. Passing H, Bablok W. A new biometrical procedure for testing the equality of measurements from two different analytical methods. J. Clin. Chem. Clin. Biochem. (1983) **21**: 709-720.
12. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th Edition, Washington, DC, AACC Press (2000).
13. Young DS. Effects of Preanalytical Variables on Clinical Laboratory Tests. 2nd Edition, Washington, DC, AACC Press (1997) **3**: 120-132.