Clinical Chemistry

2023/06/15
A93A01324DUS

ABX Pentra Amylase CP

REF A11A01628
REAGENT 1 26 mL
REAGENT 2 6.5 mL

IVD CE Rx Only

- Pentra C400

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

Diagnostic reagent for quantitative *in vitro* determination of α-Amylase in serum, plasma and urine by colorimetry.

Application Release

Serum, plasma: Amy

1.xx

Urine: Amy-U

1.xx

Intended Use [a]

ABX Pentra Amylase CP reagent is intended for the quantitative *in vitro* diagnostic determination of the activity of the enzyme amylase in human serum, plasma and urine based on an enzymatic photometric assay.
Amylase measurements are used primarily for the diagnosis and treatment of pancreatitis (inflammation of the pancreas).

Clinical Interest (1, 2)

α-Amylases are hydrolytic enzymes which break down starch into maltose. In the human body α-amylases originate from various organs: the pancreatic amylase is produced by the pancreas and released into the intestinal tract, the salivary amylase is synthesized in the salivary glands and secreted into saliva. The amylase present in the blood is eliminated through the kidney and excreted into the urine. Therefore, elevation of serum activity is reflected in a rise of urinary amylase activity.
Measurement of α-amylase in serum and urine is mainly used for the diagnosis of pancreatic disorders as well as for detecting the development of complications. In acute pancreatitis the blood amylase activity increases within few hours after onset of abdominal pain, peaks after approx. 12 hours and returns to values within the reference range at the latest after 5 days. The specificity of α-amylase for pancreatic disorders is not very high as elevated levels are measured also in various non-pancreatic diseases, e.g. parotitis and renal insufficiency. Therefore, for confirmation of an acute pancreatitis measurement of lipase should be additionally performed.

Method (3, 4)

Enzymatic photometric test, in which the substrate 4,6-ethylidene-(G7)-p-nitrophenyl-(G1)-α-D-maltoheptaoside (EPS-G7) is cleaved by α-amylases into various fragments. These are further hydrolyzed in a second step by α-glucosidase producing glucose and p-nitrophenol. The increase in absorbance represents the total (pancreatic and salivary) amylase activity in the sample.

$$5\ \text{EPS-G7} + 5\ H_2O \xrightarrow{\alpha\text{-Amylase}} 2\ \text{Ethylidene-G5} + 2\text{G2PNP} + 2\ \text{Ethylidene-G4} + 2\text{G3PNP} + \text{Ethylidene-G3} + \text{G4PNP}$$

$$2\ \text{G2PNP} + 2\ \text{G3PNP} + \text{G4PNP} + 14\ H_2O \xrightarrow{\alpha\text{-Glucosidase}} 5\ \text{PNP} + 14\ \text{G}$$

(PNP = p-Nitrophenol, G = Glucose)

Reagents [b]

ABX Pentra Amylase CP is ready-to-use.

Reagent 1 (R1):	
Good's buffer pH 7.15	0.1 mol/L
NaCl	62.5 mmol/L

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

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Reagent 1 (R1):	
$MgCl_2$	12.5 mmol/L
α-Glucosidase	≥ 2 kU/L

Reagent 2 (R2):	
Good's buffer pH 7.15	0.1 mol/L
EPS-G7	8.5 mmol/L

ABX Pentra Amylase 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. Position a protective cap ref. GBM0969 on Reagent 1 and on Reagent 2.
4. Place the cassette into the refrigerated Pentra C400 reagent compartment.

Calibrator

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

Control [c] [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)
- **Yumizen C Urine Level 1 Control** (1300023946) (not included)
  6 x 5 mL
- **Yumizen C Urine Level 2 Control** (1300023947) (not included)
  6 x 5 mL

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] [d]

- Automated clinical chemistry analyzer: Pentra C400
- Calibrator: **ABX Pentra Multical** (A11A01652)
- Controls:
  ABX Pentra N MultiControl (1300054414)
  ABX Pentra P MultiControl (1300054415)
  Yumizen C Urine Level 1 Control (1300023946)
  Yumizen C Urine Level 2 Control (1300023947)
- Standard laboratory equipment.

Specimen (5) [e]

This device intended testing population is general population.

- Serum.
- Plasma in lithium heparin.
- Urine.

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

Stability:

Serum, plasma (5)

- At 20-25°C: 7 days
- At 4-8°C: 7 days
- At -20°C: 1 year

Urine (6)

- At 20-25°C: 2 days
- At 4-8°C: 10 days
- At -20°C: 3 weeks

[c]Modification: control removed.
[d]Modification: new control.
[e]Modification: recommendation added.

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Reference Range [f]

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

Serum, plasma (7)

	Women	Men
IFCC at 37°C	< 100 U/L	< 100 U/L

Urine (8)

Spontaneously voided urine: ≤ 460 U/L
24 hours urine collection: ≤ 410 U/24h

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 C400".

Do not freeze.

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 [g]

- 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.
- Saliva and skin contain α-amylase therefore never pipette reagents by mouth and avoid skin contact with the reagents.
- **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 (9).
- 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 [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.

Serum, plasma

The performance data listed below are representative of performance on HORIBA Medical Systems.

Number of tests: 125 tests

[f]Modification: information added.
[g]Modification: general precautions modification.
[h]Modification: chapter added.

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On Board Reagent Stability

Once opened, the reagent cassette placed in the refrigerated Pentra C400 compartment is stable for 60 days.

Sample volume: 4.0 µL/test

Detection Limit [i]

The detection limit is determined according to CLSI (NCCLS), EP17-A2 protocol (10) and equals 3.27 U/L.

Limit of Quantitation [j]

The limit of quantitation is determined according to CLSI (NCCLS), EP17-A2 protocol (10) and equals 4.50 U/L.

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 U/L	CV %
Control specimen 1	74.6	0.69
Control specimen 2	180.6	0.71
Specimen 1	50.0	1.78
Specimen 2	89.2	1.18
Specimen 3	258.4	0.60

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
- 2 specimens (medium / high levels)

	Mean value U/L	CV %
Control specimen 1	76.7	2.70
Control specimen 2	184.1	1.74
Specimen 1	71.5	2.74
Specimen 2	415.0	1.73

Measuring Range [k]

The assay confirmed a measuring range from 4.5 U/L to 2000 U/L.
The measuring range is extended up to 6000 U/L with the automatic post-dilution.
The reagent linearity has been assessed up to 2000 U/L according to the recommendations found in the CLSI (NCCLS), EP06-Ed2 protocol (13).

Correlation [l]

Patient samples: Serum
Number of patient samples: 128
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 6.0 U/L to 1969.00 U/L.
The equation for the allometric line obtained using Passing-Bablok regression procedure (15) is:
Y = 1.173 X - 7.356 (U/L)
with a correlation coefficient r^2 = 0.996.

Interferences [m]

Haemoglobin:	No significant influence is observed up to 278 µmol/L (479 mg/dL).
Triglycerides:	No significant influence is observed up to a triglyceride concentration of 6.29 mmol/L (550.38 mg/dL).
Total Bilirubin:	No significant influence is observed up to 451 µmol/L (26.4 mg/dL).
Direct Bilirubin:	No significant influence is observed up to 474 µmol/L (27.7 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 10 days.
Note: A recalibration is recommended when reagent lots change, and when quality control results fall outside the range established.

[i]Modification: modification of detection limit.
[j]Modification: data added.
[k]Modification: modification of measuring range.
[l]Modification: modification of correlation.
[m]Modification: modification of interferences.

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Urine

The performance data listed below are representative of performance on HORIBA Medical Systems.

Number of tests: 125 tests

On Board Reagent Stability

Once opened, the reagent cassette placed in the refrigerated Pentra C400 compartment is stable for 60 days.

Sample volume: 4.0 µL/test

Detection Limit [i]

The detection limit is determined according to CLSI (NCCLS), EP17-A2 protocol (10) and equals 3.27 U/L.

Limit of Quantitation [j]

The limit of quantitation is determined according to CLSI (NCCLS), EP17-A2 protocol (10) and equals 4.50 U/L.

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 U/L	CV %
Control specimen 1	44.7	3.16
Control specimen 2	169.4	1.14
Specimen 1	86.4	1.65
Specimen 2	157.7	0.63
Specimen 3	286.8	0.63

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 U/L	CV %
Control specimen 1	54.0	4.29
Control specimen 2	163.5	1.51
Specimen 1	46.8	6.03
Specimen 2	135.5	3.96
Specimen 3	394.5	3.19

Measuring Range [k]

The assay confirmed a measuring range from 4.5 U/L to 2000 U/L.
The measuring range is extended up to 6000 U/L with the automatic post-dilution.
The reagent linearity has been assessed up to 2000 U/L according to the recommendations found in the CLSI (NCCLS), EP06-Ed2 protocol (13).

Correlation [l]

Patient samples: urine
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 (14).
Values ranged from 10.85 U/L to 1943.23 U/L.
The equation for the allometric line obtained using Passing-Bablok regression procedure (15) is:
Y = 1.169 X + 19.49 (U/L)
with a correlation coefficient r^2 = 0.989.

Interferences

Haemoglobin: No significant influence is observed up to 290 µmol/L (500 mg/dL).
Direct Bilirubin: No significant influence is observed up to 650 µmol/L (38 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 10 days.
Note: A recalibration is recommended when reagent lots change, and when quality control results fall outside the range established.

[i]Modification: modification of detection limit.
[j]Modification: data added.
[k]Modification: modification of measuring range.
[l]Modification: modification of correlation.

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Reference

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