Clinical Chemistry

2023/10/24
A93A00172KEN

REF A11A01645

REAGENT 2 x 20 mL

IVD CE

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

ABX Pentra CO_2 RTU

- **ABX Pentra 400**

Diagnostic reagent for quantitative *in vitro* determination of Bicarbonate / Total CO_2 in serum or plasma by colorimetry.

Application Release

Serum, plasma: CO2

3.xx

Intended Use

ABX Pentra CO_2 RTU reagent is intended for the quantitative *in vitro* diagnostic determination of carbon dioxide in human serum and plasma based on an enzymatic test using phosphoenolpyruvate (PEP), phosphoenolpyruvate carboxylase (PEPC) and an analog of NADH. Bicarbonate/carbon measurements are used in the diagnosis and treatment of numerous potentially serious disorders associated with changes in body acid-base balance.

Clinical Interest (1)

Plasmatic bicarbonates are one of the principle buffer of the organism. Their measurement is used in the diagnosis of the acid-base-balance in the blood. This balance is based on the Henderson-Hasselbach equation ($pH=pK+\log([bicarbonates]/pCO_2)$) which implies that all compensation mechanisms are intended for maintaining the relation ($[bicarbonates]/pCO_2$) constant.
Elevated and decreased values indicate disorders associated with disturbances of the metabolic and respiratory systems.

Method (2)

Enzymatic test using phosphoenolpyruvate carboxylase (PEPC) and a stable NADH analog.

$$\text{Phosphoenolpyruvate} + HCO_3^- \xrightarrow{\text{PEPC} + Mg^{2+}} \text{Oxaloacetate} + H_2PO_4^-$$

$$\text{Oxaloacetate} + \text{Cofactor red.} \xrightarrow{\text{MDH}} \text{Malate} + \text{Cofactor}$$

The reaction disturbs following equilibrium:

$$CO_2 + H_2O \longleftrightarrow H_2CO_3 \longleftrightarrow H^+ + HCO_3^-$$

(PEPC = Phosphoenolpyruvate carboxylase, MDH = Malate Dehydrogenase)

This results in a conversion of CO_2 to bicarbonate (HCO_3^-) which then is included in the reaction. Therefore the total CO_2 concentration is measured.
The decrease of reduced cofactor concentration is measured at 405 nm and is proportional to the concentration of total carbon dioxide in the sample.

Reagents

ABX Pentra CO_2 RTU is ready-to-use.

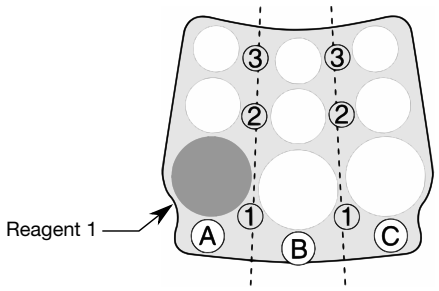
Reagent:	
Buffer pH 7.5	
Phosphoenolpyruvate (PEP)	12.5 mmol/L
Phosphoenolpyruvate carboxylase (PEPC)	> 400 U/L
Malate dehydrogenase (MDH)	> 4100 U/L
NADH analog	0.6 mmol/L
Activators, stabilizers, surfactant, preservative	

ABX Pentra CO_2 RTU should be used according to this notice. The manufacturer cannot guarantee its performance if used otherwise.

ABX Pentra CO_2 RTU

Handling

1. Transfer the necessary reagent volume for a daily workload into a 15, 10 or 4 mL reagent vial.
2. Place the vial in position 1 of one of the available areas.
   Please use one of the following:
   - a 15 mL reagent vial
   - a 10 mL reagent vial + a specific adaptor
   - a 4 mL reagent vial + a specific adaptor



3. If present, remove foam by using a plastic pipette.
4. Place the reagent rack into the refrigerated ABX Pentra 400 reagent compartment.
5. Wait for 3 hours to stabilize the reagent.

Important note: Discard the remaining reagent at the end of the day.

Calibrator

For calibration, use:
ABX Pentra CO_2 Cal (A11A01648) (not included)
3 x 3 mL

Control

For internal quality control, use:

- **ABX Pentra CO_2 Control** (A11A01650) (not included)
  3 x 3 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 [a]

- Automated clinical chemistry analyzer: ABX Pentra 400
- Calibrator: **ABX Pentra CO_2 Cal** (A11A01648)
- Control: **ABX Pentra CO_2 Control** (A11A01650)
- 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 [b]

This device intended testing population is general population.

- 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.

Stability (3, 4)**:**

- At 20 - 25°C: 1 day
- At 4 - 8°C: 7 days
- At -20°C: 2 weeks

1. Serum or plasma should be separated from cells immediately and stored at 2-8°C.
2. Exposure of samples to air should be minimized.
3. Samples should be stored tightly sealed to prevent loss of carbon dioxide and assayed as soon as possible after collection.
4. Do not use icteric samples.

Reference Range (1) [c]

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

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[a]Modification: modification of materials required.
[b]Modification: modification of "Specimen".
[c]Modification: information added.

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Adults: 22 - 29 mmol/L.

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 [d]

Stability before opening:

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

Stability after opening:

Stable for 28 days at 2-8°C if closed immediately and contamination is avoided. Store protected from light.

Do not freeze.

Waste Management [e]

- 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 vials 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 ABX Pentra 400

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: < 10%.

Serum, plasma

The performance data listed below have been obtained on the ABX Pentra 400 analyzer.

Number of tests: approximately 200 tests

On Board Reagent Stability

Use fresh reagent each day. Discard the remaining reagent in container after use.

Sample volume: 3.0 µL/test

[d]Modification: modification of storage and stability.
[e]Modification: modification of waste management.
[f]Modification: general precautions modification.
[g]Modification: chapter added.

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Detection Limit [h]

The detection limit is determined according to CLSI (NCCLS), EP17-A2 protocol (6) and equals 1.45 mmol/L.

Limit of Quantitation [i]

The limit of quantitation is determined according to CLSI (NCCLS), EP17-A2 protocol (6) and equals 1.8 mmol/L.

Accuracy and Precision

Repeatability (within-run precision)

Repeatability according to the recommendations found in the Valtec protocol (7) with samples tested 20 times:

- 1 control
- 3 specimens (low / medium / high levels)

	Mean value mmol/L	CV %
Control specimen	20.44	1.25
Specimen 1	10.93	0.78
Specimen 2	21.30	0.51
Specimen 3	32.03	0.66

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):

- 1 control
- 2 specimens (low / high levels)

	Mean value mmol/L	CV %
Control specimen	20.75	4.8
Specimen 1	9.53	7.7
Specimen 2	31.57	5.9

Measuring Range [j]

The assay confirmed a measuring range from 1.8 mmol/L to 60.8 mmol/L.
The measuring range is extended up to 121.6 mmol/L with the automatic post-dilution.
The reagent linearity has been assessed up to 60.8 mmol/L according to the recommendations found in the CLSI (NCCLS), EP06-Ed2 protocol (9).

Correlation [k]

Patient samples: Serum and plasma
Number of patient samples: 125
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 2.20 mmol/L to 59.58 mmol/L.
The equation for the allometric line obtained using Passing-Bablok regression procedure (11) is:
Y = 0.9688 x – 1.153 (mmol/L)
with a correlation coefficient r^2 = 0.964.

Interferences [l]

Haemoglobin:	No significant influence is observed up to 195 µmol/L (336 mg/dL).
Triglycerides:	No significant influence is observed up to a triglyceride concentration of 6.17 mmol/L (539.88 mg/dL).
Total Bilirubin:	No significant influence is observed up to 100 µmol/L (5.85 mg/dL).
Direct Bilirubin:	No significant influence is observed up to 370 µmol/L (21.6 mg/dL).

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

Calibration Stability [m]

The reagent is calibrated on Day 0. The calibration stability is checked by testing 1 control specimen.
The calibration stability is 24 hours.
Note: A recalibration is recommended when reagent lots change, and when quality control results fall outside the range established.

Reference

1. Müller-Plathe O. Acid base balance and blood gases. In: Thomas L., editor. Clinical laboratory diagnostics. 1st ed. Frankfurt: T.H. Books Verlagsgesellschaft (1998): 318-329.
2. Norris KA, Atkinson AR, Smith WG. Colorimetric Enzymatic Determination of Serum Total Carbon Dioxyde as Applied to the Vickers Multichannel 300 Discrete Analyzer. Clin. Chem. (1975) **21**: 1093-1101.

[h]Modification: modification of detection limit.
[i]Modification: modification of quantitation limit.
[j]Modification: modification of measuring range.
[k]Modification: modification of correlation.
[l]Modification: modification of interferences.
[m]Modification: modification of calibration stability.

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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).
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