Clinical Chemistry

2023/10/05
A93A01236JEN

ABX Pentra CK-MB RTU

REF A11A01643
REAGENT 1 1 x 20 mL
REAGENT 2 1 x 5 mL

IVD CE

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

- Pentra C200

Diagnostic reagent for quantitative *in vitro* determination of CK-MB in serum by colorimetry.

Application Release

Serum: CKMB (not for use in the USA)

01.xx

Intended Use (not for use in the USA)

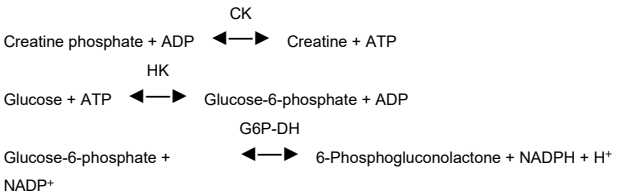
ABX Pentra CK-MB RTU reagent is intended for the quantitative *in vitro* diagnostic determination of CKMB in serum by colorimetry. Measurements of Creatine Kinase are used in the diagnosis and treatment of myocardial infarction and muscle diseases such as progressive, Duchenne-type muscular dystrophy.

Clinical Interest (1, 2)

Creatine kinase (CK) is an enzyme which consists of isoenzymes mainly of the muscle (CK-M) and the brain (CK-B). CK exists in serum in dimeric form as CK-MM, CK-MB, CK-BB and as macroenzyme. Elevated CK values are observed in cardiac muscle damages and in skeletal muscle diseases. Measurement of CK is used especially in conjunction with CK-MB for diagnosis and monitoring of myocardial infarction.
The use of CK-MB to measure cardiac muscle damage has been used for decades and have been progressively removed by the use of troponin as a gold standard. Nevertheless, in some countries, mostly developing countries, where the troponin testis not available mainly for cost issues, CK MB remains a primary indicator of the muscle damage.
In those countries the determination of CK-MB by activity measurements is not recommended if mass assay technique is available.

Method [a]

History: the method for the determination of the activity of creatine kinase (CK) using a coupled enzymatic reactions was initially described by Oliver (3) and then modified by Rosalky (4).
The DGKC (German Society of Clinical Chemistry) (5) and the IFCC (International Federation of Clinical Chemistry) (6) standardized the method thereafter recommending the reversibility of the oxidation of the CK and the activation of this one by N-acetylcysteine (NAC). The IFCC confirmed this one and extended the method to 37°C in 2002 (7), which is the method used here.
Optimized UV test according to DGKC and IFCC for CK with inhibition of CK-M isoenzymes by monoclonal antibodies (5, 8).
CK-MB consists of the subunits CK-M and CK-B. Specific antibodies against CK-M inhibits the complete CK-MM activity (main part of the total CK activity) and the CK-M subunit of CK-MB. Only CK-B activity is measured, which is half of the CK-MB activity.



Reagents

ABX Pentra CK-MB RTU is ready-to-use.

[a]Modification: information added.

QUAL-QA-WORI-5541 Rev.2

ABX Pentra CK-MB RTU

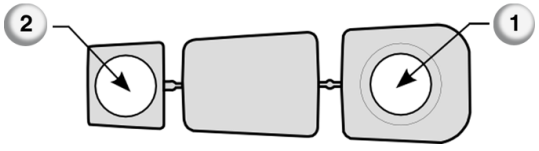
Reagent 1:	
Imidazole	120 mmol/L
Glucose	25 mmol/L
N-Acetylcysteine (NAC)	25 mmol/L
Magnesium acetate	12.5 mmol/L
EDTA-Na_2	2 mmol/L
NADP	2.5 mmol/L
Hexokinase (HK)	≥ 5 kU/L
Monoclonal antibodies against human CK-M; inhibiting capacity	2500 U/L

Reagent 2:	
Imidazole	90 mmol/L
Creatine phosphate	150 mmol/L
ADP	10 mmol/L
AMP	28 mmol/L
Diadenosine pentaphosphate	50 µmol/L
Glucose-6-phosphate dehydrogenase (G6P-DH)	≥ 15 kU/L
Stabilizers	

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

Handling

1. Identify the cassette by using dedicated reagent stickers with barcode (601).
2. Transfer the reagent R1 into compartment 1 (30 mL capacity) of the cassette 30/10 provided (see diagram below).
3. Transfer the reagent R2 into compartment 2 (10 mL capacity) of the cassette 30/10 provided (see diagram below).



4. If present, remove foam by using a plastic pipette.
5. Place the reagent cassette at an available position on the reagent tray in the refrigerated Pentra C200.

Calibrator

N/A: factor mode.

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

Specimen [c]

This device intended testing population is general population.

- Serum.

Stability (9)

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

[b]Modification: control removed.
[c]Modification: modification of specimen stability.

ABX Pentra CK-MB RTU

Reference Range [a] (1)

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

< 24 U/L (37°C).

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. Store protected from light.

Stability after opening:

Stable up to the expiry date on the label if stored at 2-8°C, closed immediately and contamination is avoided. Store protected from light.

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 [d]

- This reagent is for professional *in vitro* diagnostic use only.
  For laboratory use.
- For prescription use only.
- This reagent is classified as hazardous in compliance with regulation (EC) N°.1272/2008.
- **Reagent 1 and 2 (R1 and R2):**
  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 (10).
- **Reagent 1 (R1):**
  Danger
  H360D: May damage the unborn child.
  P201: Obtain special instructions before use.
  P202: Do not handle until all safety precautions have been read and understood.
  P280: Wear protective gloves/protective clothing/eye protection/face protection.
  P308 + P313: IF exposed or concerned: Get medical advice/attention.
  P405: Store locked up.
  P501: Dispose of contents and container in accordance with all local, regional, national and international regulations.
  Contains: Imidazole
- **Reagent 2 (R2):**
  Danger
  H360D: May damage the unborn child.
  P201: Obtain special instructions before use.
  P202: Do not handle until all safety precautions have been read and understood.
  P280: Wear protective gloves/protective clothing/eye protection/face protection.
  P308 + P313: IF exposed or concerned: Get medical advice/attention.
  P405: Store locked up.
  P501: Dispose of contents and container in accordance with all local, regional, national and international regulations.
  Contains: Imidazole
- 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.

[a]Modification: information added.
[d]Modification: general precautions modification.

ABX Pentra CK-MB RTU

- 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 [e]

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

Serum

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

Number of tests: approximately 85 tests

On Board Reagent Stability [f]

The reagent cassette placed in the refrigerated Pentra C200 compartment is stable for 20 days.

Sample volume: 6 µL/test

Limit of Quantitation [g]

The limit of quantitation is determined according to CLSI (NCCLS), EP17-A2 protocol (11) and equals 8 U/L.

Accuracy and Precision

Repeatability (within-run precision)

Repeatability according to the recommendations found in the Valtec protocol (12) with samples tested 20 times:

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

	Mean value U/L	CV %
Control specimen	35.86	2.49
Specimen 1	57.08	2.76
Specimen 2	141.48	1.42
Specimen 3	241.42	1.10

Reproducibility (total precision)

Reproducibility according to the recommendations found in the CLSI (NCCLS), EP5-A2 protocol (13) with samples tested in duplicate for 20 days (2 series per day):

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

	Mean value U/L	CV %
Control specimen	37.6	3.19
Specimen 1	55.3	2.86
Specimen 2	141.2	1.98
Specimen 3	242.7	2.03

Measuring Range [h]

The assay confirmed a measuring range from 8 U/L to 300.0 U/L.
The reagent linearity has been assessed up to 300.0 U/L according to the recommendations found in the CLSI (NCCLS), EP06-Ed2 protocol (14).

Correlation [i]

Patient samples: Serum
Number of patient samples: 40
Specimens are correlated with a commercial reagent taken as reference according to the recommendations found in the CLSI (NCCLS), EP09c protocol (15).
Values ranged from 17.05 U/L to 279.45 U/L.
The equation for the allometric line obtained using Passing-Bablok regression procedure (16) is:
Y = 1.076 X + 2.842 (U/L)
with a correlation coefficient r^2 = 0.997.

Interferences [j]

Haemoglobin:	Do not use hemolysed samples.
Triglycerides:	No significant influence is observed up to a triglyceride concentration of 6.77 mmol/L (592.4 mg/dL).
Total Bilirubin:	No significant influence is observed up to 421.1 µmol/L (24.6 mg/dL).

[e]Modification: chapter added.
[f]Modification: on board reagent stability modification.
[g]Modification: data added.
[h]Modification: modification of measuring range.
[i]Modification: modification of correlation.
[j]Modification: modification of interferences.

ABX Pentra CK-MB RTU

Direct Bilirubin: No significant influence is observed up to 350 µmol/L (20.5 mg/dL).
The presence of Sulfasalazine or Sulfapyridine in sample can cause false results.

Other interferences:

- CK-MM isoenzyme is inhibited at 99% (in-house study).
- As the methodology used measures the activity of the monomer CKB, an overestimation of the CK-MB activity could occur in case of (17, 18, 19, 20):
  - elevated CK-BB activity
  - macro form of CK-BB (CK-BB bound to IgG and polymeric complex of mitochondrial CK)

Other limitations are given by Young as a list of drugs and preanalytical variables known to affect this methodology (21, 22).

Calibration Stability [k]

The reagent is calibrated on Day 0. The calibration stability is checked by testing 1 control specimen.
The calibration stability is 20 days.
Note: A recalibration is recommended when reagent lots change, and when quality control results fall outside the range established.

Reference

1. Stein W. Creatine kinase (total activity), creatine kinase isoenzymes and variants. In: Thomas L, ed. Clinical laboratory diagnostics. Frankfurt: TH-Books Verlagsgesellschaft (1998): 71-80.
2. Moss DW, Henderson AR. Clinical enzymology. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company (1999): 617-721.
3. Oliver JT. A spectrophotometric method for the determination of creatine phosphokinase and myokinase. Biochem. J. (1955) **61**: 116-122.
4. Rosalky SB, J. Lab. Clin. Med. (1967) **69**: 696-705.
5. Recommendations of the German Society for Clinical Chemistry. Standardization of methods for the estimation of enzyme activities in biological fluids: Standard method for the determination of creatine kinase activity. J. Clin. Chem. Clin. Biochem. (1977) **15**: 255-260.
6. Horder M, Elser RC, Gerhardt M and al. Approved Recommendation on IFCC Methods for the Measurement of Catalytic Concentration of Enzymes. Part 7. IFCC Method for Creatine Kinase. Eur. J. Clin. Chem. Clin. Biochem. (1991) **29**: 435-456.
7. Schumann G and al., IFCC Primary Reference procedures for the Measurement of Catalytic Activity Concentration of Enzymes at 37°C. Part 2. Reference Procedures for the Measurement of Catalytic Concentration of Creatine Kinase, Clin Chem Lab Med. (2002) **40** (6): 635-642.
8. Würzburg U, Hennrich N, Orth HD, Lang H. Quantitative determination of creatine kinase isoenzyme catalytic concentrations in serum using immunological methods. J. Clin. Chem. Clin. Biochem. (1977) **15**: 131-137.
9. Guder WG, Zawta B. The Quality of Diagnostics Samples. Samples: From the Patient to the Laboratory. 1st ed. Guder WG, Narayanan S, Zawta B. (WHILEY-VCH, Darmstadt, Germany) (2001): 24.
10. Council Directive (2000/54/EC). Official Journal of the European Communities. No. L262 from October 17, 2000: 21-45.
11. Evaluation of detection capability for clinical laboratory measurement procedures. Approved Guideline, 2nd ed., CLSI (NCCLS) document EP17-A2 (2012) **32** (8).
12. Vassault A, Grafmeyer D, Naudin C et al. Protocole de validation de techniques (document B). Ann. Biol. Clin. (1986) **44**: 686-745.
13. Evaluation of Precision Performance of Quantitative Measurement Method. Approved Guideline, CLSI (NCCLS) document EP5-A2 (2004) **24** (25).
14. Evaluation of Linearity of Quantitative Measurement Procedures. 2nd Edition, CLSI (NCCLS) guideline EP06-Ed2 (2020) **40** (16).
15. Measurement Procedure Comparison and Bias Estimation Using Patient Samples. Approved Guideline, 3rd ed., CLSI (NCCLS) document EP09c (2018) **38** (12).
16. 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.
17. Neumeier D, Prellwitz W, Determination of creatine kinase isoenzyme MB activity in serum using immunological inhibition of creatine kinase M subunit activity. Activity kinetics and diagnostic significance in myocardial infarction, Clin Chim Acta. (1976) **73** (3): 445-51.
18. Ljungdahl L, Gerhardt W. Creatine kinase isoenzyme variants in human serum, Clin. Chem. (1978) **24** (5): 832-834.

[k]Modification: modification of calibration stability.

ABX Pentra CK-MB RTU

19. Urdal P, Landaas S, Macro Creatine kinase BB in serum, and some data on its prevalence, Clin. Chem. (1979) **25** (3): 461-465.
20. Wu AHB, Bowers GNJr. Evaluation and comparison of immunoinhibition and immunoprecipitation methods for differentiating MB from BB and macro forms of creatine kinase isoenzymes in patients and healthy individuals, Clin. Chem. (1982) **28** (10): 2017-2021.
21. Young DS. Effects of Drugs on Clinical Laboratory Tests. 4th Edition, Washington, DC, AACC Press (1997) **3**: 143-163.
22. Young DS. Effects of Preanalytical Variables on Clinical Laboratory Tests. 2nd Edition, Washington, DC, AACC Press (1997) **3**: 120-132.