Manual Procedure

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Cat. No. 17791 R1 1 x 24 ml
For 30 tests R2 1 x 6 ml

Creatine Kinase-MB (CK- MB)

UV kinetic method activated NAC

Based on IFCC recommendations

Liquid Reagents

Test principle

Total serum or plasma creatine kinase (CK) activity represents CK-MM and CK-MB isoenzyme activity, the contribution of the CK-BB isoenzyme normally being undetectable. The CK-MB reagent contains antibodies
directed against the CK-M subunit, giving complete inhibition of CK-MM and partial inhibition of CK-MB (inhibition of CK-M fraction without affecting the CK-B fraction).The CK-B fraction accounts for one half the activity of CK-MB; it is determined using the following series of reactions:

Creatine phosphate + ADP $\xrightarrow{CK}$ Creatine + ATP

ATP + D-Glucose $\xrightarrow{HK}$ ADP+D-Glucose-6--phosphate

D-Glucose-6-phosphate+NADP$^+$ $\xrightarrow{G\text{-}6\text{-}PDH}$ D-Gluconate-6-phosphate + NADPH + H^+

The remaining CK-B catalyzes the reversible phosphorylation of ADP, in the presence of creatine phosphate, to form ATP and creatine.
The auxiliary enzyme hexokinase (HK) catalyzes the phosphorylation of glucose by the ATP formed to produce ADP and glucose -6- phosphate (G6P). The G6P is oxidized to 6-phosphogluconate with the concomitant production of NADPH. The rate of NADPH formation, measured at 340 nm, is directly proportional to serum CK-B activity.

Concentrations in the test

Reagent R1		
N-Acetylcystein (NAC)	30	mmol/L
ADP	5.88	mmol/L
NADP	3	mmol/L
AMP	7.2	mmol/L
Diadenosine (5) pentaphosphate	10	µmol/L
G-6-PDH (Glucose-6-phosphate-dehydrogenase)	≥ 2800	U/L
Hexokinase	≥ 4000	U/L
Anti-human polyclonal CK-MB antibody	Sufficient to inhibit up to 1500 U/L CK-MM	
Stabilizer, preservative		
Reagent R2		
D-Glucose	40	mmol/L
Magnesium acetate	15.3	mmol/L
Creatine phosphate	150	mmol/L
EDTA	2.6	mmol/L
Stabilizer, preservative		

Stability and preparation of working reagent

Reagent R1: *liquid*.
Reagent R2: *liquid*.
All reagents are stable up to expiry date given on the label when stored at +2 ➔ +8 °C.

Working Reagent: (4+1)
Avoid direct exposure to light .
Mix 4 volumes of bottle R1 with 1 volume of bottle R2.
Stability: 14 days at 2 - 8 °C.

Note: Don't use if bacterial contamination is evident (turbidity), and the working reagent has an absorbance greater than 0.700 at 340 nm against water.

Specimen collection and handling

1. Serum, plasma collected in heparin or EDTA is the specimen of choice.
2. Store sample in refrigerator (2 - 8 °C), but no longer than one week. Freezing of samples at - 20 °C results in minimal loss of activity.
3. Avoid exposure of samples to strong light.

Quality control

Meditrol N Cat. No. 15171
Meditrol P Cat. No. 15181

Procedure

Wavelength	Hg 340 nm (334 - 365 nm)
Spectrophotometer	340 nm
Cuvette	1 cm light path
Temperature	37°C
Measurement	against air or distilled water
Reaction	kinetic – increase

Assay: incubate Working Reagent at 37°C:

Sample	50 µl
Working Reagent	1000 µl
Mix, incubate for 5 min. at 37°C. Read change in the absorbance per 1 min. for 3 min. Determine the mean absorbance change per 1 min. (ΔA/min).	

Factors & Calculation at 37°C

Wavelength	**CK-MB** (U/L)
334 nm	6796 XΔA
340 nm	6666 XΔA
365 nm	12000 XΔA

$$\text{CK-MB activity \%} = \frac{\text{CK-MB}_{\text{activity}}}{\text{Total CK}_{\text{activity}}} \times 100$$

Note: It is recommended that each laboratory (as per instrument performance) could make its own factor (F) by the use of a calibrator according to the following formula:

$$F = \frac{Conc_{calibrator}}{\Delta A/\min_{\text{Calibrator}}}$$

Linearity

If the assay of the total CK as reported above is greater than 1000 U/L at 37°C, dilute the sample appropriately with sodium chloride solution 0.9 % before assay of CK-MB. Multiply the result by the dilution factor to obtain the corrected value of the isoenzyme.

Interferences

1. Hemolyzed samples should not be used since erythrocytes contain contaminants and enzymes that will interfere with the assay.
2. Bilirubin level up to 20 mg/dl has been found to have a negligible effect on this assay.
3. Young, et al. lists a number of substances that will affect the assay.
4. The method will also measure CK-BB isoenzyme present in serum. The activity of this isoenzyme is usually negligible, however, if a significant amount of CK-BB activity is present the CK-MB activity will be overestimated.
5. A macro form of BB (immunoglobulin complexed) has been observed that will be measured as B in this assay. If the measured CK-B activity is greater than 20 % of the total CK activity the presence of macro BB should be suspected.

Precautions

1. Reagent may be irritating to skin, flush skin with water if contacted.
2. This reagent contains sodium azide as a preservative. Don't ingest. May react with lead and copper plumbing to form highly explosive metal azide. Upon disposal, flush with a large volume of water to prevent azide build up.

Reference range

CK-MB activity is up to 25 U/L (at 37°C).

A ratio between CK-MB and total CK activities above 4 % should be considered suspicious, even though it could be caused by extensive skeletal muscle injury. Any ratio between 5.0 – 25 % is consistent with acute myocardial infarction. It is highly recommended that each laboratory establish its own expected range.

References

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6. Rosalki, S.B., J. Lab. Clin. Med. 69:696 (1967).
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