CLINICAL SIGNIFICANCE

Creatine Kinase consists of the M and B subunits and is found in the cardiac muscle. CK-MB activity is significantly increased in myocardial infarction and this increase is very specific for the laboratory diagnosis of myocardial infarction.

METHOD PRINCIPLE

The enzymatic immunoinhibition of the M subunit is applied. The anti-CK-M antibody present in the CK-MB reagent inhibits the catalytic activity of the M subunit of CK-MB enzyme. The observed activity is only due to the B subunit of the enzyme. The activity is measured as the increase of the signal at 340 nm observed following the following reactions:

- Phosphocreatine + ADP + CK enzyme \( \mathbb{K} \) Creatine + ATP
- ATP + D-Glucose \( \mathbb{K} \) Glucose-6-Phosphate + ADP
- Glucose-6-Phosphate + NAD+ \( \mathbb{K} \) 6-Phosphoglycerol + NADH + H+

CK : Creative Kinase
HK : Hexokinase
G6PDH : Glucose 6-Phosphate Dehydrogenase

The increase of absorption at 340 nm is proportional to the CK-MB concentration in the sample.

PREPARATION

Reagents are liquid, ready-to-use, and are placed on the corresponding positions of the analyzer. Vials bear barcode for recognition by BECKMAN COULTER AU Series analyzers.

REAGENT DETERIORATION

The reagent should not be used:
- When it does not exhibit specified linearity or recovery of control values lies outside the acceptable range after recalibration.
- After prolonged exposure to high temperature.

SHELF LIFE

Unopened, the reagent is stable to the stated expiry date when stored at 2 to 8 °C. Once opened, it remains stable for 3 weeks when stored refrigerated on the analyzer.

SAMPLE

Lipemic, hemolyzed and strongly icteric samples should be avoided. Especially, hemolyzed samples should not be used because ATP, adenylic acid kinase and glucose-6-phosphate dehydrogenase are abundant in red blood cells and they may interfere severely in the reaction. CK-MB activity remains steady for 24 hours in room temperature (15 – 20°C) and for 7 days at 2 – 6°C, or up to 1 year at –20°C. Samples should be tested shortly after collection.

CALIBRATION

Determination takes place in MB Calibration Mode. The calibration factor is derived by the mean of at least three separate analysis series, on different days, in single point calibration mode (MB). MEDICON provides the MEDICON CKMB Control (1578-029) for further investigation. A new calibrator should be used for each series of analyses. Recalibration should be repeated after major maintenance is performed on the analyzer or after a critical part is replaced or when a significant shift in control values occurs.

QUALITY CONTROL

MEDICON suggests the MEDICON CK-MB CONTROL (1578-029). Control values and limits shall fall within acceptable intervals adjusted to the requirements of each laboratory. Target values for CK-MB should be verified with the corresponding working protocol. Results outside the specified values even after recalibration could be due to reagent deterioration, unsuitable storage conditions or control deterioration, instrument malfunction, or error during test procedure.

MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT

CK-MB calibrator
Quality control material
Autoimmunochemistry analyzer
Common laboratory equipment

REFERENCE INTERVALS

Serum:
- CK: MB < 24 U/L when total CK is within normal limits.
- CK: Women: < 167 U/L (37°C)
- Men: < 210 U/L (37°C)

A CK-MB fraction more than 6% of the total activity is regarded as diagnostic for myocardial infarction. A fraction less than 6% indicates skeletal muscle damage. A fraction bigger than 25% may indicate the presence of Macro-MB requiring further investigation. If CK-MB is normal for a patient with suspected heart attack, repeat testing with new sample collection 4 hours later.

Each laboratory should determine its own expected values as dictated by good laboratory practice.

WASTE DISPOSAL

This product contains sodium azide (NaN₃), which forms sensitive explosive compounds with copper or lead. Flush waste pipes with water after the disposal of undiluted reagent in order to avoid azide build up in the drain pipes.

SPECIFIC PERFORMANCE CHARACTERISTICS

Linearity

The assay is linear up to 2000 U/L. When values exceed this range, samples should be diluted accordingly.

Sensitivity

The lowest detectable level of CK-MB is estimated at 1.5 U/L.

The lowest detection limit (LDL) is defined as the lowest concentration of analyte that is distinguishable from zero. A sample free of analyte is assayed 20 times within the assay and the LDL is calculated as the absolute mean plus three standard deviations.

Precision

- Interference

  - Lipemic: insignificant up to 900 mg/dL
  - Hemoglobin: insignificant up to 50 mg/dL
  - Non Con. Bilirubin: insignificant up to 40 mg/dL
  - Con. Bilirubin: insignificant up to 40 mg/dL
  - Acetic acid: insignificant up to 3 mg/dL

- Method Comparison

  A comparison was performed between this reagent and another commercially available product. The results were as follows:

  $$ Y = 1.0114X - 0.5613 $$

  R₄ = 0.9911

  N₅₀ sample range: 10 – 117 U/L

METHOD LIMITATIONS

This method also measures the CK-BB isoenzyme. Although its activity is usually negligibly, the presence of CK-BB may give falsely elevated CK-MB values. A macromorph of the CK-BB isoenzyme may be present during the determination of CK-MB. If the activity of the CK-BB isoenzyme surpasses the 20% of total CK activity, then the presence of CK-BB macromorph may be supposed. Overestimation of CK-MB may occur in cases of CK macromorph presence in the sample.

ATP, adenylic acid kinase and glucose-6-phosphate dehydrogenase are abundant in red blood cells and may interfere severely in the reaction, so the use of hemolysed samples should be avoided.

The anti-CK-MM antibody is sufficient to inhibit CK-MM activity by 99.75% up to 2000 U/L and by 99% up to 8000 U/L. In order to measure CK-MB in samples with total CK greater than 8000 U/L, a suitable dilution should be made to ensure total inhibition of CK-MB subunit.

BIBLIOGRAPHY

10. Wu AHB, Bowens CN Jr.: “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”.

SYMBOLS

- Temperature Limits: 2°C-8°C
- Interfering Substances: Glucose, Uric Acid, Hemoglobin
- Catalogue Number: (ISO 15223/rev. EN980/I SO7000).
- Manufacturer: (ISO 15223/rev. EN980/I SO7000).
- Production Date: (ISO 15223/rev. EN980/I SO7000).
- For in vitro use: (ISO 15223/rev. EN980).