Clinical significance
CK-MB is composed of two moieties CK-M (from muscles) and CK-B (from brain). CK-MB is usually present in serum at low concentration; it is increased after an acute infarct or myocardium and later descends at normal levels. Rarely, it can also be increased in skeletal muscle damage. Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

Principle
A specific antibody inhibits the CK-M moiety without affecting the CK-B moiety. The CK-B fraction accounts for one half of the activity of CK-MB; it is determined by the NAC-activated method.

The rate of NADPH formation, measured photometrically, is proportional to the catalytic concentration of CK-B present in the samples.

Reagent composition

Reagent 1
- L-Tris-HCl pH 7.4........................................125 mmol/L
- D-Glucose..................................................25 mmol/L
- Na-acetylcysteine............................25 mmol/L
- Magnesium acetate..............................12,5 mmol/L
- NADP...........................................................2,52 mmol/L
- EDTA..........................................................2,92 mmol/L
- Hexokinase (HK)........................................0,6 mmol/L
- G-6-PDH....................................................22800 U/L

Reagent 2
- ADP...........................................................15,2 mmol/L
- AMP...........................................................25 mmol/L
- D-ribose-5-P..............................................103 mmol/L
- G-6-PDH....................................................22800 U/L

Reagent Control
- Lyophilized human serum.........................2 mL
- Value ( assay at 37°C) indicated on label

Anti human polyclonal CK-M antibody (sheep) is sufficient to inhibit up to 2000 U/L of CK-M moiety.

For in vitro diagnostic use only.

Precautions
The control is prepared from human sera, which have been tested and are found to be non-reactive for HBsAg, HCV and HIV antibodies. However, all human specimens should be considered potentially infectious.

Preparation

Working reagent: Mix 4 volumes of R1 with 1 volume of R2. The stability of the working reagent is 5 days at 2-8°C or 24 hours at room temperature (15-25°C).

Control: Dissolve the contents in 2 ml of distilled water. Cap vial and mix gently to dissolve the contents. Stability: 3 days at 2-8°C or 1 month at –20°C. Bring at room temperature for about 30 min before use.

Storage and stability
All the components of the kit are stable at 2-8°C up to the date of expiration as specified, when stored tightly closed, protected from light and contaminations prevented during their use.

The reagent should be a clear solution. If turbidity or precipitation has occurred or if blank absorbance at 340 nm ≥ 0,1°C, the reagent should be discarded.

Additional equipment
- Spectrophotometer or colorimeter measuring at 340 nm
- Thermostatic bath at 25°C, 30°C or 37°C (± 0,1°C)
- Matched cuvettes 1,0 cm light path

Samples
- Serum free of hemolysis or heparin plasma: stability 7 days at 2-8°C, protected from light.
- CK-MB activity decreases a 10% after 24 hours at 4°C or 1 hour at 25°C.

Procedure for manual method
1. Dissolve the contents in 1 ml of distilled water. Cap vial and mix gently to dissolve the contents.
2. Stability: 3 days at 2-8°C or 24 hours at room temperature (15-25°C).
3. Working reagent is 5 days at 2-8°C or 24 hours at room temperature (15-25°C).

Procedure for CYANStart
1. Dissolve the contents in 1 ml of distilled water. Cap vial and mix gently to dissolve the contents.
2. Stability: 1 month at –20°C or 1 month at 2-8°C.
3. Working reagent is 5 days at 2-8°C or 24 hours at room temperature (15-25°C).

Working reagent

<table>
<thead>
<tr>
<th>Working reagent</th>
<th>Sample</th>
<th>1,0 ml</th>
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<tbody>
<tr>
<td>Sample</td>
<td>40 µL</td>
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</table>

Procedure
1. Wavelength 340 nm; Temperature 25, 30, 37°C; Cuvette 1 cm light path.
2. Adjust the instrument to zero with distilled water or air.
3. Pipette into a cuvette:
   - Working reagent 1,0 ml
   - Mix and aspirate immediately. The following program will run 3 minutes of incubation time and 2 minutes of kinetic measurement.

Calculation

Mean (U/l) 24,95 66  25 74
Intra-assay (n=20) Inter-assay (n=20)
CV (%) 10,36 4,59  9,80 2,62
SD 2,58 3,03  2,45 1,84

Sensitivity: 1 U/L = 0.001055 ΔAbs/min
Accuracy: Results obtained using CYPRESS DIAGNOSTICS reagents did not show systematic differences when compared with other commercial reagents.

Reference values

<table>
<thead>
<tr>
<th>CK-MB</th>
<th>25°C</th>
<th>30°C</th>
<th>37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10 U/L</td>
<td>&lt;/=</td>
<td>&lt;/=</td>
<td>&lt;/=</td>
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<tr>
<td>&lt; 15 U/L</td>
<td>15</td>
<td>15</td>
<td>15</td>
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<tr>
<td>&lt; 24 U/L</td>
<td>24</td>
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Limitation of the procedure
The method will also measure any CK-BB isoenzyme present in serum. The activity of the isoenzyme is negligible. However, if a significant amount of CK-BB activity is present, the CK-MB activity will be underestimated.

Interferences
No interferences were observed with glucose until 7 g/l, hemoglobin until 6 g/l and triglycerides until 800 mmol/l. A list of drugs and other interfering substances with CK-MB determination has been reported by Young et al.

Bibliography