LIQUID LINE

CK-MB NAC UV AA

UV method for determination of the Creatine Kinase MB isoenzyme in serum or plasma through monoclonal antibodies anti CK-M

SUMMARY

Creatine Kinase (CK) is an intramuscular enzyme constituted by a subunit M (muscle) and other subunit B (brain) which combine giving place to isoenzymes CK-MM (muscular), CK-BB (brain) and CK-MB (myocardial).
Serum increase of CK and of CK-MB is an indicator of myocardial injury. After an acute myocardial infarction, in approximately 55% of the cases, the highest increase of CK and CK-MB is simultaneously produced, while in 45% of the cases the highest increase of CK-MB precedes the one total CK.

PRINCIPLE

The method is based on the specific inhibition of the CK-M subunits with monoclonal antibodies anti CK-M. The antibodies inhibit not only the MM isoenzyme but also the M subunits corresponding to CK-MB. Subunits B are determined by using a reactive system based on an analytical technique optimized by the IFCC, with N-acetyl-cysteine as activator, adding monoclonal antibodies anti-CK-M.

PROVIDED REAGENTS

A. Reagent A: imidazole buffer solution.
B. Reagent B: solution containing creatine phosphate, anti-CK M antibodies and reactive components in sufficient containing enough quantities to obtain the following final concentrations:

Imidazole	100 mmol/l; pH 6.7
Creatine phosphate	30 mmol/l
ADP	2 mmol/l
Glucose	20 mmol/l
NADP	2 mmol/l
Hexokinase	≥ 2500 U/l
Glucose-6-phosphate dehydrogenase	≥ 2000 U/l
Magnesium acetate	10 mmol/l
AMP	5 mmol/l
Di (adenosine-5´) pentaphosphate	10 umol/l
N-acetyl-cysteine (NAC)	20 mmol/l

Antibodies capable of inhibiting 1000 U/l of CK-M.
Control: vial containing lyophilized human CK-MB (see attached table for theoretic value).

NON-PROVIDED REAGENTS

Saline Solution (9 g/dl sodium chloride).
Distilled water.

INSTRUCTIONS FOR USE

Provided Reagents: ready to use. They may be used separately or as **Monoreagent**, mixing 5 parts Reagent A with 1 part Reagent B (e.g. 5 ml Reagent A + 1 ml Reagent B).
Control: open the vial carefully trying to not spill the content. Reconstitute with the distilled water volume stated in the label. Cap and wait for 5 minutes. Dissolve the content of the vial completely by inversion. The reconstituted CK-MB Control is treated in the same way as an unknown sample.

WARNINGS

Reagents are for “in vitro” diagnostic use.
Control has been tested for hepatitis B virus surface antigen, hepatitis C virus and antibodies to HIV 1/2, being found non-reactive. Nonetheless, it should be handled as infectious material.
Use the reagents according to the working procedures for clinical laboratories.
The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: stable at 2-10ºC until the expiration date stated on the box. Once opened, they should not remain outside the refrigerator for extended periods of time. Avoid contamination.
Monoreagent (premixed)**:** stable at 2-10ºC for 20 days after reconstitution date.
Reconstituted Control: stable at 2-10ºC for up to 3 days, for 2 days at 25ºC or for 3 months in freezer (-20ºC). Do not freeze and thaw repeatedly.

INSTABILITY OR DETERIORATION OF REAGENTS

When spectrophotometer has been set to zero with distilled water, absorbance readings of Monoreagent that are higher than 0.500 O.D. (at 340 nm) indicate deterioration.

SAMPLE

Serum or plasma
a) Collection: obtain in the usual way.
b) Additives: heparin or EDTA. The use of Wiener lab.’s **Anticoa-gulante W** is recommended.
c) Known interfering substances: no interferences have been observed by bilirubin up to 390 mg/l (39 mg/dl), triglycerides up to 3.0 g/l (300 mg/dl) nor hemoglobin up to 0.06 g/dl (60 mg/dl) (mild hemolysis). Sera with visible hemolysis should not be used as they produce falsely increased values.
See Young, D.S. in References for effect of drugs on the present method.
d) Stability and storage instructions: sample should be

fresh. At 2-10°C, the sample loses up to 10% of the enzymatic activity in one day.

REQUIRED MATERIAL (non-provided)
- Spectrophotometer.
- Micropipettes and pipettes for measuring the stated volumes
- Water bath at the temperature indicated under PROCEDURE.
- Stopwatch.

ASSAY CONDITIONS
- Wavelength: 340 nm (Hg 334 or 366).
- Reaction temperature: 25, 30 or 37°C. Select temperature according to instrument. See the REFERENCE VALUES corresponding to each temperature.
- Reaction time: 6 minutes
- Sample and reagent volumes: may vary proportionally (e.g. 100 ul sample + 2.5 ml Monoreagent or 20 ul sample + 500 ul Monoreagent).

PROCEDURE

MONOREAGENT TECHNIQUE

Set the instrument to zero O.D. with distilled water. In a cuvette at the selected temperature (25, 30 or 37°C) place:

Monoreagent	1 ml
Pre-incubate a few minutes. Then add:	
Sample	40 ul

Mix immediately by inversion. Wait for 10 minutes. Adjust absorbance to a reference reading and simultaneously start stopwatch. Measure absorbance every minute for 3 minutes. Determine average change in Absorbance/min (ΔA/min), subtracting each reading from the previous one and averaging these values. Use this mean for calculations.

CALCULATIONS

CK-MB (U/l) = ΔA/min x factor
Measure at 340 nm: CK-MB (U/l) = ΔA/min x 8,254
Measure at Hg 334 : CK-MB (U/l) = ΔA/min x 8,414
Measure at Hg 366: CK-MB (U/l) = ΔA/min x 14,858
The calculation factors mentioned above, already include the correction needed to convert the value of CK-B into CK-MB.

QUALITY CONTROL METHOD

Each time the test is running, analyze two levels of a quality control material (**CK-MB Control**) with known CK-MB activities.

REFERENCE VALUES

Temperature	25°C	30°C	37°C
Values	≤ 10 U/l	≤ 16 U/l	≤ 25 U/l

SI SYSTEM UNITS CONVERSION

CK-MB (U/l) x 0.017 = CK-MB (ukat/l)

INTERPRETATION OF RESULTS

A high probability of myocardial damage exists if the following conditions are simultaneously met:

1- Total CK activity exceeds the following normal ranges:

Temperature	25°C	30°C	37°C
Men	10-80 U/l	15-130 U/l	24-195 U/l
Women	10-70 U/l	15-110 U/l	24-170 U/l

The possibility of a recent infarction exists if myocardial damage is suspected and values are below the normal range. In this case repeat testing after 4 hours.
2- CK-MB activity exceeds normal values. See REFERENCE VALUES.
3- The CK-MB percentage is found between the 6-20% of the total CK value.
If the percentage is bellow 6% there is probably damage to the skeletal muscle. If the percentage is over 20% of the total CK value the presence of a macro kind of CK (atypical CK) which is not inhibited by the anti-CK-M antibodies, can be suspected.
The atypical CK presence may be determined by:
a) Persistence for more than 48 hours (the CK-MB decays approximately at 30-48 hours after the onset of the infarction).
b) Stability when treating the sample at 40°C during 20 minutes.
c) Electrophoretic analysis (a band between MM and MB isoenzymes is obtained).

PROCEDURE LIMITATIONS

See Known interfering substances under SAMPLE.
Samples with total CK activity over 1000 U/l should be diluted with saline solution. The obtained result should be multiplied by the dilution performed.

PERFORMANCE

a) Reproducibility: CLSI protocol EP15-A was applied. Three concentration levels were tested, in replicates by four, during 5 days. With the obtained data, total and intra-assay precision were calculated.

Intra-assay precision (n = 20)

Level	S.D.	C.V.
41 UI/l	± 0.69 UI/l	1.7 %
232 UI/l	± 2.55 UI/l	1.1 %

Total precision (n = 20)

Level	S.D.	C.V.
41 UI/l	± 0.98 UI/l	2.4 %
232 UI/l	± 3.94 UI/l	1.7 %

b) Linearity: the reaction is linear up to 500 UI/l.
c) Analytical sensitivity: depends on the photometer used and wavelength. In spectrophotometer at 340 nm with 1 cm optical length square cuvettes, for ΔA/min of 0.001, the minimum detectable activity change will be of 8 U/l.

PARAMETERS FOR AUTOANALYZERS

For programming instructions check the user's manual of the autoanalyzer in use.

WIENER LAB PROVIDES

60 ml (Cat. Nº 1271361): 1 x 50 ml A
1 x 10 ml B
1 x C → 2 ml

60 ml (Cat. Nº 1009333): 3 x 17 ml A
1 x 10 ml B
1 x C → 2 ml

120 ml (Cat Nº 1009249): 5 x 20 ml A
1 x 20 ml B
1 x C → 2 ml

120 ml (Cat Nº 1009608): 2 x 50 ml A
1 x 20 ml B
1 x C → 2 ml

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SYMBOLS

The following symbols are used in the packaging for Wiener lab. diagnostic reagents kits.

CE	This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices
EC REP	Authorized representative in the European Community
IVD	"In vitro" diagnostic medical device
	Contains sufficient for <n> tests
	Use by
	Temperature limitation (store at)
	Do not freeze
	Biological risks
→	Volume after reconstitution
Cont.	Contents
LOT	Batch code
	Manufactured by:
	Harmful
	Corrosive / Caustic
	Irritant
	Consult instructions for use
Calibr.	Calibrator
CONTROL	Control
CONTROL +	Positive Control
CONTROL −	Negative Control
REF	Catalog number

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