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CK-MB STAT

cobas®

CK-MB - the MB isoenzyme of creatine kinase (STAT "Short Turn Around Time")

REF	Σ	SYSTEM
05957648 190	100	Elecsys 2010 **cobas e** 411 **cobas e** 601 **cobas e** 602

English

Intended use

Immunoassay for the in vitro quantitative determination of the MB isoenzyme of creatine kinase in human serum and plasma.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Creatine kinase (CK) is a dimeric enzyme which occurs in four different forms: a mitochondrial isoenzyme and the cytosolic isoenzymes CK-MM (muscle type), CK-BB (brain type) and CK-MB.[1,2]

The determination of CK-MB mass in serum is an important element in the diagnosis of myocardial ischemia, e.g. in acute myocardial infarction, myocarditis, etc.[1,2] CK-MB is detectable in the blood about 3-8 hours after the onset of cardiac symptoms and can remain detectable over a lengthy period of time, depending on the course of the condition.[1]

CK-MB may also appear in other clinical conditions, e.g. in rhabdomyolysis and stroke.[1] Within the scope of laboratory diagnostics, the determination of total CK, troponin T and/or myoglobin can contribute to the differentiation of these clinical pictures.

The sensitivity of a CK-MB determination is dependent upon the time at which a sample was taken. Follow-up assays are therefore meaningful.[1,3]

The Elecsys CK-MB STAT assay employs two different monoclonal antibodies directed against human CK-MB.

Test principle

Sandwich principle. Total duration of assay: 9 minutes.

Elecsys 2010 and **cobas e** 411 analyzers:

- 1st incubation: 15 µL of sample, a biotinylated monoclonal anti-CK-MB antibody, and a monoclonal CK-MB-specific antibody labeled with a ruthenium complex[a)] react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

cobas e 601 and **cobas e** 602 analyzers:

- During a 9 minute incubation, antigen in the sample (15 µL), a biotinylated monoclonal anti-CK-MB antibody, a monoclonal CK-MB-specific antibody labeled with a ruthenium complex and streptavidin-coated microparticles react to form a sandwich complex, which is bound to the solid phase.

All analyzers:

- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The reagent rackpack is labeled as CKMBSTAT.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-CK-MB-Ab~biotin (gray cap), 1 bottle, 9 mL:
Biotinylated monoclonal anti-CK-MB antibody (mouse) 1.2 mg/L; phosphate buffer 100 mmol/L, pH 7.0; preservative.

R2 Anti-CK-MB-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 9 mL:
Monoclonal anti-CK-MB antibody (mouse) labeled with ruthenium complex 1.2 mg/L; phosphate buffer 100 mmol/L, pH 7.0; preservative.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in automatically from the respective reagent barcodes.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on the analyzers	8 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, Na-heparin, K_2-EDTA and K_3-EDTA plasma.

Criterion: Recovery within 80-120 % of value from single serum/plasma pairs or slope 0.9-1.1 + intercept within < ± 0.5 x Limit of Detection (LoD) + coefficient of correlation > 0.95.

Stable for 4 hours at 18-23 °C, 8 hours at 2-8 °C, 3 months at -20 °C. Freeze only once.

CK-MB stability is extremely temperature-dependent. A CK-MB decrease of > 10 % can occur after the sample has stood for 1 hour at 32 °C.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

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Materials required (but not provided)

- REF 05957656190, CK-MB STAT CalSet, for 4 x 1 mL
- REF 04917049190, PreciControl Cardiac II, for 2 x 2 mL each of PreciControl Cardiac II 1 and 2
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- REF 03609987190, Diluent MultiAssay, 2 x 16 mL sample diluent
- General laboratory equipment
- Elecsys 2010 or **cobas e** analyzer

Accessories for Elecsys 2010 and **cobas e** 411 analyzers:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- REF 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500316, Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles prior to use and the reading in of the test-specific parameters via the reagent barcode take place automatically. No manual input is necessary. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Calibration

Traceability: The Elecsys CK-MB STAT assay is traceable to the Abbott IMx CK-MB assay and linearized using human recombinant CK-MB[4] from Seradyn.

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Cardiac II.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in ng/mL or µg/L).

Limitations - interference

The assay is unaffected by icterus (bilirubin < 581 µmol/L or < 34 mg/dL), hemolysis (Hb < 0.621 mmol/L or < 1.0 g/dL), lipemia (Intralipid < 1500 mg/dL), IgG < 7.0 g/dL, IgM < 1.0 g/dL, IgA < 1.6 g/dL, albumin < 20 g/dL and biotin (< 123 nmol/L or < 30 ng/mL).

Criterion: Recovery with a standard deviation ≤ 0.4 ng/mL of initial value at concentrations between 0.3-5 ng/mL; recovery within ± 20 % of initial value at concentrations > 5 ng/mL.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL and samples from dialysis patients.

There is no high-dose hook effect at CK-MB concentrations up to 5000 ng/mL.

In vitro tests were performed on 51 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

0.3-300 ng/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 0.3 ng/mL. Values above the measuring range are reported as > 300 ng/mL (or up to 600 ng/mL for 2-fold diluted samples).

Lower limits of measurement

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ)

Limit of Blank = 0.1 ng/mL

Limit of Detection = 0.3 ng/mL

Limit of Quantitation = 1 ng/mL with a total allowable error of ≤ 20 %

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable error of ≤ 20 %.

Dilution

Samples with CK-MB concentrations above the measuring range can be diluted with Diluent MultiAssay. The recommended dilution is 1:2 (either automatically by the Elecsys 2010 or **cobas e** analyzers or manually). On the **cobas e** 601 and **cobas e** 602 analyzers, Diluent Universal can also be used. The concentration of the diluted sample must be > 50 ng/mL.

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After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzers, the Elecsys 2010 and **cobas e** software automatically takes the dilution into account when calculating the sample concentration.

Expected values

The values below were obtained in two studies (Kiel I and Kiel II) using the Elecsys CK-MB assay (4th generation). The calculation is based on samples from 879 apparently healthy volunteers (463 women, 416 men).

	N	Median ng/mL	97.5th percentile ng/mL	99th percentile ng/mL
Women	463	1.39	3.61	4.88
Men	416	1.72	4.87	6.22

When myocardial infarction is suspected the diagnostic strategy proposals in the consensus document of European and American cardiologists should in general be followed.[5]

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, samples and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplication each for 21 days (n = 84). The following results were obtained:

Elecsys 2010 and **cobas e** 411 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	0.621	0.018	2.9	0.035	5.7
Human serum 2	5.46	0.066	1.2	0.135	2.5
Human serum 3	29.5	0.397	1.3	1.24	4.2
Human serum 4	93.5	1.25	1.3	3.86	4.1
Human serum 5	301	4.46	1.5	10.0	3.3
PreciControl CARD1	4.44	0.059	1.3	0.115	2.6
PreciControl CARD2	57.9	0.828	1.4	1.76	3.0

cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	0.644	0.018	2.8	0.020	3.1
Human serum 2	5.34	0.061	1.1	0.075	1.4
Human serum 3	27.3	0.289	1.1	0.885	3.2
Human serum 4	89.2	0.946	1.1	2.25	2.5
Human serum 5	283	2.19	0.8	6.09	2.2
PreciControl CARD1	4.27	0.050	1.2	0.059	1.4
PreciControl CARD2	54.3	0.503	0.9	0.723	1.3

Method comparison

A comparison of the Elecsys CK-MB STAT assay (y) with the Elecsys CK-MB STAT assay - previous version (x) using clinical samples gave the following correlations:

Number of samples measured: 196

Passing/Bablok[6]	Linear regression
y = 1.048x - 0.326	y = 1.085x - 0.915
τ = 0.975	r = 0.999

The sample concentrations were between 0.3 and 300 ng/mL.

Analytical specificity

For the monoclonal antibodies used, the following cross-reactivities were found:

CK-MM none, CK-BB 0.1 %.

References

1 Rozenman Y, Gotsman MS. The earliest diagnosis of acute myocardial infarction. Annu Rev Med 1994;45:31-44.

2 Adams JE, Abendschein DR, Jaffe AS. Biochemical markers of myocardial injury: Is MB creatine kinase the choice for the 1990s? Circulation 1993;88:750-763.

3 Apple FS. Diagnostic markers for detection of acute myocardial infarction and reperfusion. Laboratory Medicine 1992;23(5):297-322.

4 Christenson RH, Vaidya H, Landt Y, et al. Standardization of Creatine Kinase-MB (CK-MB) Mass Assays: The Use of Recombinant CK-MB as a Reference Material. Clin Chem 1999;45(9):1414-1423.

5 Thygesen K, Alpert JS, White HD. Universal Definition of Myocardial Infarction. J Am Coll Cardiol 2007;50:2173-2195.

6 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing

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