VIDAS® CK-MB (CKMB)

IVD

VIDAS CK-MB is an automated quantitative test for use on the VIDAS family instruments for the quantitative measurement of the MB isoenzyme of human creatine kinase in human serum or plasma (lithium heparin or EDTA), using the ELFA technique (Enzyme Linked Fluorescent Assay).

SUMMARY AND EXPLANATION

Creatine kinase (CK) is a key enzyme of muscle metabolism. It is present in all tissues, but in variable concentrations and in different isoforms (CK-MM, CK-MB and CK-BB) (1). In skeletal muscle, creatine kinase is essentially found in the MM isoform (1). The BB isoform is found predominantly in the brain and the MB fraction is found principally in the heart tissue (1, 2).

In the absence of any major muscular trauma, an elevation of serum CK-MB concentration indicates necrotic injury to the heart occurring as a consequence of myocardial infarction. Generally, CK-MB is detectable five hours after the onset of chest pains and peak concentrations are often reached eleven to eighteen hours after the infarction (3, 4).

There exists, however, a certain number of other situations, pathological or otherwise, in which an elevation of CK-MB can be observed, such as Duchenne's disease, muscular hyperactivity, hypo- and hyperthermia, and myocarditis (2).

The VIDAS CK-MB assay aids in diagnosing acute coronary syndromes.

PRINCIPLE

The assay principle combines the enzyme immunoassay sandwich method with a final fluorescent detection (ELFA) (5, 6, 7).

The Solid Phase Receptacle (SPR®) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips.

All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times.

A pre-wash step prepares the SPR for the reaction. The sample is then transferred into the well containing the serum diluent. The sample/serum diluent mixture is cycled in and out of the SPR. Unbound components are eliminated during the washing steps. The conjugate (alkaline phosphatase-labeled anti-CK-MM antibody fragment) is cycled in and out of the SPR. Unbound components are eliminated during the washing steps.

During the final detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone), the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of CK-MB present in the sample. At the end of the assay, the results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out.

CONTENT OF THE KIT (30 TESTS) - RECONSTITUTION OF REAGENTS

30 CKMB strips	STR	Ready-to-use.
30 CKMB SPRs 1 x 30	SPR®	Ready-to-use. Interior of SPRs coated with monoclonal anti-CK-MB immunoglobulins (mouse).
CKMB control 1 x 3 ml (lyophilized)	C1	Reconstitute with 3 ml of reconstitution solvent. Wait for 10 to 15 minutes, then mix. After reconstitution, stable for 24 hours at 2-8°C: In aliquots and frozen at -25 ± 6°C, stable until the expiration date on the kit. Avoid successive freeze/thaw cycles. Thaw at room temperature (18-25°C). Protein base (BSA) + chemical stabilizers + human CK-MB. MLE data indicate the confidence interval in ng/mL ("Control C1 Dose Value Range").
CKMB calibrator 1 x 3 ml (lyophilized)	S1	Reconstitute with 3 ml of reconstitution solvent. Wait for 10 to 15 minutes, then mix. After reconstitution, stable for 24 hours at 2-8°C. In aliquots and frozen at -25 ± 6°C, stable until the expiration date on the kit. Avoid successive freeze/thaw cycles. Thaw at room temperature (18-25°C). Protein base (BSA) + chemical stabilizers + human CK-MB. MLE data indicate the concentration in ng/mL (“Calibrator (S1) Dose Value") and the confidence interval in "Relative Fluorescence Value ("Calibrator (S1) RFV Range”).
Reconstitution solvent 1 x 20 ml (liquid)	R1	Ready to use. Demineralized water + 0.9 g/l sodium azide.
Specifications for the factory master data required to calibrate the test: • MLE data (Master Lot Entry) provided in the kit, or • MLE bar codes printed on the box label.		
1 Package Insert provided in the kit or downloadable from www.biomerieux.com/techlib.		

The SPR

The SPR is coated during production with monoclonal anti-CK-MB immunoglobulins (mouse). Each SPR is identified by the CKMB code. Only remove the required number of SPRs from the pouch a**nd carefully reseal the pouch after opening.**

The strip

The strip consists of 10 wells covered with a labeled foil seal. The label comprises a bar code which mainly indicates the assay code, kit lot number and expiration date. The foil of the first well is perforated to facilitate the introduction of the sample. The last well of each strip is a cuvette in which the fluorimetric reading is performed. The wells in the center section contain the various reagents required for the assay.

Description of the CKMB strip

Wells	Reagents
1	Sample.
2	Serum diluent: Tris-NaCl (0.05 mol/l, pH 7.4), protein and chemical stabilizers, 0.9 g/l sodium azide (300 µl).
3	Wash buffer: Tris-NaCl (0.05 mol/l, pH 7.4), protein stabilizers, 0.9 g/l sodium azide (600 µl).
4 - 5 - 7 - 8	Wash buffer: Tris-NaCl (0.05 mol/l, pH 7.4), surfactant, 0.9 g/l sodium azide (600 µl).
6	Conjugate: alkaline phosphatase-labeled anti-CK-MM polyclonal antibody Fab’ fragment (goat), Tris-NaCl buffer (0.05 mol/l, pH 6), protein stabilizers, 0.9 g/l sodium azide (400 µl).
9	Empty well.
10	Cuvette with substrate: 4-Methyl-umbelliferyl phosphate (0.6 mmol/l) + diethanolamine (DEA*) (0.62 mol/l, or 6.6%, pH 9.2) + 1 g/l sodium azide (300 µl).

* Signal Word: **DANGER**

Hazard statement

H318 : Causes serious eye damage.

Precautionary statement

P280 :Wear protective gloves/protective clothing/eye protection/face protection.
P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

For further information, refer to the Material Safety Data Sheet.

MATERIALS AND DISPOSABLES REQUIRED BUT NOT PROVIDED

- Pipette with disposable tip to dispense 3 ml and 250 µl.
- Powderless, disposable gloves.
- For other specific materials and disposables, please refer to the Instrument User's Manual.
- Instrument of the VIDAS family.

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use only.**
- **For professional use only.**
- **This kit contains products of human origin. No known analysis method can totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (see Laboratory Biosafety Manual - WHO - Geneva - latest edition).**
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest or inhale).
- Do not use SPRs if the pouch is pierced.
- Do not use visibly deteriorated STRs (damaged foil or plastic).
- Do not use reagents after the expiration date indicated on the label.
- Do not mix reagents (or disposables) from different lots.
- Use **powderless gloves** as powder has been reported to cause false results for certain enzyme immunoassay tests (8).
- Kit reagents contain sodium azide which can react with lead or copper plumbing to form explosive metal azides. If any liquid containing sodium azide is disposed of in the plumbing system, drains should be flushed with water to avoid build-up.
- The optical cuvette with substrate (well 10) contains an irritant agent (6.6% diethanolamine). Refer to the hazard statements "H" and the precautionary statements "P" above.
- Spills should be wiped up thoroughly after treatment with liquid detergent or a solution of household bleach containing at least 0.5% sodium hypochlorite. See the User's Manual for cleaning spills on or in the instrument. Do not autoclave solutions containing bleach.
- The instrument should be regularly cleaned and decontaminated (see the User's Manual).

STORAGE CONDITIONS

- Store the VIDAS CK-MB kit at 2-8°C.
- **Do not freeze reagents, with the exception of calibrators and controls after reconstitution.**
- **Store all unused reagents at 2-8°C.**
- After opening the kit, check that the SPR pouch is correctly sealed and undamaged. If not, do not use the SPRs.
- **Carefully reseal the pouch with the desiccant inside after use to maintain the stability of the SPRs and return the complete kit to 2-8°C.**
- If stored according to the recommended conditions, all components are stable until the expiration date indicated on the label. Refer to the kit composition table for special storage conditions.

SPECIMENS

Specimen type and collection

Serum or plasma (lithium heparin or EDTA).
It is recommended that each laboratory checks the compatibility of collection tubes used.
None of the following factors have been found to significantly influence this assay:
- hemolysis (after spiking samples with hemoglobin: 0 to 5 g/l (monomer)),
- lipemia (after spiking samples with lipids: 0 to 34.2 mmol/l equivalent in trigycerides),
- bilirubinemia (after spiking samples (naturally icteric plasma) with bilirubin: 0 to 850 µmol/l).

However, it is recommended not to use samples that are clearly hemolyzed, lipemic or icteric and, if possible, to collect a new sample.

Specimen stability

Samples can be stored at 2-8°C in stoppered tubes for up to 48 hours.
If longer storage is required, freeze the sera or plasma at at -25 $\pm$ 6 C.
Avoid successive freezing and thawing.
Thaw at room temperature (18-25°C).
A study performed on frozen samples over a period of 5 months showed that the quality of results is not affected.

INSTRUCTIONS FOR USE

For complete instructions, see the User's Manual.

Reading Master lot data

Before each new lot of reagents is used, enter the specifications (or factory master data) into the instrument using the master lot entry (MLE) data.

If this operation is not performed **before initiating the tests**, the instrument will not be able to print results.

Note: the master lot data need only be entered once for each lot.

It is possible to enter MLE data **manually or automatically** depending on the instrument (refer to the User's Manual).

Calibration

Calibration, using the calibrator provided in the kit, must be performed each time a new lot of reagents is opened, after the master lot data have been entered. Calibration should then be performed every 14 days. This operation provides instrument-specific calibration curves and compensates for possible minor variations in assay signal throughout the shelf-life of the kit.
The calibrator, identified by S1, must be tested **in duplicate** (see User's Manual). The calibrator value must be within the set RFV (Relative Fluorescence Value). If this is not the case: recalibrate.

Test procedure

1. **Only remove the reagents required from the refrigerator.**
2. Use one “CKMB” strip and one “CKMB” SPR for each sample, control or calibrator to be tested. **Make sure the storage pouch has been carefully resealed after the required SPRs have been removed**.
3. The test is identified by the “CKMB” code on the instrument. The calibrator must be identified by "S1", and tested **in duplicate**. If the control is to be tested, it should be identified by "C1".
4. If necessary, clarify samples by centrifugation.
5. Mix the calibrator, controls and samples using a vortex-type mixer (for serum or plasma separated from the pellet).
6. **For this test, the calibrator, control, and sample test portion is 250 µl.**
7. Insert the “CKMB” SPRs and “CKMB” strips into the instrument. Check to make sure the color labels with the assay code on the SPRs and the Reagent Strips match.
8. Initiate the assay as directed in the User's Manual. All the assay steps are performed automatically by the instrument.
9. Reclose the vials and return them to the required temperature after pipetting.
10. The assay will be completed within approximately 30 minutes. After the assay is completed, remove the SPRs and strips from the instrument.
11. Dispose of the used SPRs and reagent strips into an appropriate recipient.

RESULTS AND INTERPRETATION

Once the assay is completed, results are analyzed automatically by the computer. Fluorescence is measured twice in the Reagent Strip’s reading cuvette for each sample tested. The first reading is a background reading of the substrate cuvette before the SPR is introduced into the substrate. The second reading is taken after incubating the substrate with the enzyme remaining on the interior of the SPR. The RFV (Relative Fluorescence Value) is calculated by subtracting the background reading from the final result. This calculation appears on the result sheet.

The results are automatically calculated by the instrument using calibration curves which are stored in memory (4-parameter logistic model) and are expressed in ng/ml.
The measurement range for VIDAS CK-MB is 0.8-300 ng/ml. Samples with CK-MB concentrations greater than 300 ng/ml must be retested after dilution 1/4 in human serum with a CK-MB concentration < 0.8 ng/ml.
If the dilution factor has not been entered when the Work List was created (see User's Manual), multiply the result by the dilution factor to obtain the sample concentration.
Interpretation of test results should be made taking into consideration the patient's history, and the results of any other tests performed.

QUALITY CONTROL

A positive control is included in each VIDAS CK-MB kit.
This control must be performed immediately after opening a new kit to check that reagent performance has not been altered. Each calibration must also be checked using this control. The instrument will only be able to check the control value if it is identified by C1.
Results cannot be validated if the control value deviates from the expected values.

Note

It is the responsibility of the user to perform Quality Control in accordance with any local applicable regulations.

LIMITATIONS OF THE METHOD

Interference may be encountered with certain sera containing antibodies directed against reagent components. For this reason, assay results should be interpreted taking into consideration the patient history, and the results of any other tests performed.

RANGE OF EXPECTED VALUES

These figures are given as a guide. It is recommended that each laboratory establishes its own reference values from a rigorously selected population.
Depending on the patients tested, differences in the expected values may be found

The assay of ponderal CK-MB can be completed with the assay of total CK using a relative index (RI):

$$RI\ (\%) = \frac{\text{CK-MB (ng/ml)}}{\text{Total CK (U/l)}} \times 100$$

It is recommended that each laboratory establishes its own reference values for the calculation of the relative index (RI) due to the differences between the total CK methods and the levels of CK-MB obtained for different populations. As an indication, for a reference population of 119 blood donors, the upper limit of the 95% reference range of CK-MB was determined as 5.1 ng/ml.

PERFORMANCE

Studies performed with the VIDAS CK-MB assay gave the following results:

Measurement range

The measurement range of the VIDAS CK-MB reagent is 0.8-300 ng/ml.

Analytical detection limit

Defined as the smallest concentration of CK-MB which is significantly different from the zero concentration with a probability of 95%: **0.8 ng/ml**.

Hook effect

No hook effect was observed up to CK-MB concentrations of 10,000 ng/ml.

Precision

Within-run reproducibility:

Three samples were tested 30 times in the same run.

Samples	E1	E2	E3
Number of measurements	30	30	30
Mean concentration (ng/ml)	3.73	11.94	94.79
CV (%)	6.16	3.42	3.29

Between-run reproducibility:

Three samples were tested singly in 23 different runs on the same VIDAS instrument.

Samples	E1	E2	E3
Number of measurements	23	23	23
Mean concentration (ng/ml)	2.89	12.49	114.34
CV (%)	5.13	4.95	3.13

Specificity

No cross-reactivitiy was observed with CK-MM and CK-BB for tested concentrations of up to 10,000 ng/ml.

Accuracy

Dilution test

3 human serum samples were diluted in a weak serum. The ratio of the mean concentration measured over the expected concentration is expressed as a mean recovery percentage.

Sample	Dilution factor	Expected concentration (ng/ml)	Measured concentration (ng/ml)	Mean recovery percentage
PS 51	1/1	127.71	127.71	100
	1/2	63.86	66.88	105
	1/4	31.93	34.16	107
	1/8	15.96	16.32	102
	1/16	7.98	8.95	112
	1/32	3.99	4.17	104
PS 54	1/1	150.27	150.27	100
	1/2	75.14	93.24	124
	1/4	37.57	43.35	115
	1/8	18.78	22.07	117
	1/16	9.39	10.96	117
	1/32	4.70	5.70	121
PS 55	1/1	196.57	196.57	100
	1/2	98.29	108.00	110
	1/4	49.14	52.68	107
	1/8	24.57	26.09	106
	1/16	12.29	13.29	108
	1/32	6.14	7.05	115

Comparison with another test method

Correlation was established between the VIDAS CK-MB kit and another commercially available kit (X) using an enzyme immunoassay technique:

The allometric curve equation obtained is:
VIDAS CK-MB = 1.02 X + 0.33 r = 0.99 (n = 94)

WASTE DISPOSAL

Dispose of used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.
It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES

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6. VAIDYA H.C., BEATTY B.G. Eliminating interference from heterophilic antibodies in a two-site immunoassay for creatine kinase MB by using F(ab')2 conjugate and polyclonal mouse IgG. Clin. Chem., 1992, 38 (**9**), 1737-1742.
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INDEX OF SYMBOLS

Symbol	Meaning
	Catalog number
	*In Vitro* Diagnostic Medical Device
	Manufacturer
	Temperature limit
	Use by date
	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Date of manufacture

WARRANTY

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REVISION HISTORY

Change type categories :

N/A	Not applicable (First publication)
Correction	Correction of documentation anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user

Note: *Minor typographical, grammar, and formatting changes are not included in the revision history.*

Release date	Part Number	Change Type	Change Summary
2015/01	07709J	Administrative	INDEX OF SYMBOLS REVISION HISTORY
		Technical	CONTENT OF THE KIT (30 TESTS) – RECONSTITUTION OF REAGENTS WARNINGS AND PRECAUTIONS INSTRUCTIONS FOR USE

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