CK-MB ASSAY PROCEDURE

CK-MB QUANTITATIVE DETERMINATION
IN HUMAN SERUM AND PLASMA BY THE ACCESS® IMMUNOASSAY SYSTEMS

Annual Review:
Refer to coversheet in front of method

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This document is provided as an aid to writing laboratory procedures following NCCLS guidelines but does not include all activities identified in NCCLS GP2-A4 Clinical Laboratory Technical Procedure Manuals; Approved Guideline – Fourth Edition. Each laboratory is responsible for ensuring their procedures are comprehensive and complete.

Beckman Coulter, Inc. • Fullerton, CA 92835
**Principle**

**Principles of the Procedure**
The Access CK-MB assay is a two-site immunoenzymatic (“sandwich”) assay. Patient sample is added to a reaction vessel with mouse monoclonal anti-human CK-MB antibody-alkaline phosphatase conjugate and paramagnetic particles coated with mouse monoclonal anti-human CK-BB. Human serum CK-MB binds to the anti-CK-MB conjugate and is immobilized on the paramagnetic particle coated with anti-CK-BB. The CK-MB in the human serum or plasma binds to the immobilized anti-CK-BB on the solid phase by the subunit B epitope (common to CK-BB and CK-MB isoforms), while the mouse anti-CK-MB conjugate reacts specifically with the serum or the plasma CK-MB (no reaction with CK-MM or CK-BB isoforms). After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos® 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of CK-MB in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

**Summary and Explanation**
CK-MB is one of the three tissue isoforms (with CK-BB and CK-MM) of creatine kinase (CK). CK is the principal enzyme of muscular metabolism, which catalyses the reversible reaction of creatine phosphorylation by adenosine triphosphate (ATP). CK-MB is made up of two sub-units (MW= 40,000 each): M sub-unit, expressed in muscle, and B sub-unit, expressed in brain.

CK-MB isoenzyme is located primarily in the myocardium, representing 20% of the total CK activity (1,2). Amounts greater than 5% can be found in the prostate, spleen or skeletal muscle, where quantities of CK-MB may change as a function of the muscle type (3,4). After an acute myocardial infarction (AMI), CK-MB appears in the circulation, reflecting damage to the myocardium. CK-MB rises rapidly to peak levels (within 12 hours) then declines to normal levels (36–72 hours). This pattern of rising and falling CK-MB values, along with evolutionary changes in the ECG and a history of chest pain, is generally considered diagnostic of AMI. Measurements of CK-MB can also aid in the non-invasive assessment of the efficacy of myocardial reperfusion following thrombolytic therapy. Elevated levels of CK-MB are also associated with skeletal muscle trauma, but don’t have the rise and fall characteristics of CK-MB levels in AMI (5,6).

Immuno-inhibition technology was originally used to measure CK-MB activity, which was compared with the measurement of total CK activity (ratio CK-MB/CK) (7,8). However, the presence of CK-BB, adenylate cyclase(AK) and atypical forms of CK(macro-CK), which are not neutralized by anti-M antibodies, can occasionally cause the overestimation of CK-MB results (9).

Today, many immunoenzymatic techniques measuring CK-MB mass (ng/mL) correlate well with the measurement of CK-MB activity, without the interference of CK-BB, macro-CK, and AK (10,11).

**Specimen Collection**

A. Lithium heparin plasma is the recommended sample. Serum and plasma (heparin or EDTA) are acceptable samples. Sample types should not be used interchangeably during serial sampling. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and at times, lot to lot.

B. Observe the following recommendations for handling, processing, and storing blood samples (12):

1. Collect all blood samples observing routine precautions for venipuncture.
2. Allow serum samples to clot completely before centrifugation.
3. Keep tubes stoppered at all times.
4. Within two hours after centrifugation, transfer at least 500 µL of cell-free sample to a storage tube. Tightly stopper the tube immediately.

5. Store samples tightly stoppered at room temperature (15 to 30°C) for no longer than eight hours.

6. If the assay will not be completed within eight hours, refrigerate the samples at 2 to 8°C.

7. If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -20°C or colder.

8. Thaw samples only once.

C. Use the following guidelines when preparing specimens:

1. Ensure residual fibrin and cellular matter has been removed prior to analysis.

2. Follow blood collection tube manufacturer’s recommendations for centrifugation.

D. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot-to-lot.

E. Do not thaw in water bath. Mix gently by inversion and centrifuge after thawing prior to sample analysis. Avoid assaying lipemic, icteric or hemolyzed samples.

Reagents and Equipment

Beckman Coulter, Inc.
4300 N. Harbor Blvd.
Fullerton, CA  92835

A. R1: Access CK-MB Reagent Pack
   Cat. No.386371: 100 determinations, 2 packs, 50 tests/pack.

   Provided ready to use. Store upright and refrigerate at 2 to 10°C. Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument. Stable until the expiration date stated on the label when stored at 2 to 10°C. Stable at 2 to 10°C for 56 days after initial use. Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range. If the reagent pack is damaged (i.e. broken elastomer), discard the pack. All antisera are polyclonal unless otherwise indicated.

   1. R1a: Paramagnetic particles coated with goat anti-biotin antibodies and biotinylated anti-human CK-BB mouse monoclonal antibodies suspended in buffered solution, with bovine serum albumin (BSA), 0.2% ProClin® 950 and < 0.1% sodium azide.

   2. R1b: Purified mouse IgG and purified goat IgG in buffered solution with BSA, 0.1% ProClin 300, and < 0.1% sodium azide.

   3. R1c: Mouse monoclonal anti-human CK-MB antibody alkaline phosphatase conjugate in buffered solution with BSA, 0.1% ProClin 300, and < 0.1% sodium azide.

B. Access CK-MB Calibrators
   Cat. No. 386372: S0-S5, 2.0 mL/vial

   Quantitative assay calibration is the process by which samples with known analyte concentrations (i.e. assay calibrators) are tested like patient samples to measure the response. The mathematical
relationship between the measured responses and the known analyte concentrations establishes the calibration curve. This mathematical relationship, or calibration curve, is used to convert RLU (Relative Light Unit) measurements of patient samples to specific quantitative analyte concentrations.

Provided ready to use. Store upright and refrigerate at 2 to 10°C. Mix contents by gently inverting before use. Avoid bubble formation. Stable until the expiration date stated on the label when stored at 2 to 10°C. Stable at 2 to 10°C for 60 days after initial use. Signs of possible deterioration are control values out of range. Refer to calibration card for exact concentrations.

Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

1. S0: Buffered BSA matrix with 0.02% Cosmocil*** CQ and < 0.1% sodium azide. Contains 0.0 ng/mL of recombinant CK-MB.

2. S1–S5: Recombinant CK-MB at levels of approximately 3, 10, 30, 100, and 300 ng/mL respectively in buffered BSA matrix with 0.02% Cosmocil CQ and < 0.1% sodium azide.

3. Calibration Card: 1

C. Access Substrate
Cat. No. 81906: 4 x 130 mL

Provided ready to use. Refer to the following chart for storage conditions and stability. An increase in substrate background measurements may indicate instability.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Storage</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unopened</td>
<td>2 to 8°C</td>
<td>Until expiration date stated on the label</td>
</tr>
<tr>
<td>Equilibration prior to use (unopened)</td>
<td>15 to 30°C (room temperature)</td>
<td>Minimum 18 hours Maximum 14 days</td>
</tr>
<tr>
<td>In use (opened)</td>
<td>Internal substrate supply position</td>
<td>Maximum 5 days</td>
</tr>
<tr>
<td>In use (opened)</td>
<td>External fluids tray substrate position</td>
<td>Maximum 14 days</td>
</tr>
</tbody>
</table>

R2 Substrate: Lumi-Phos 530 (buffered solution containing dioxetane Lumigen* PPD, fluorescer, and surfactant).

Refer to the appropriate system manuals and/or Help system for detailed instructions.

D. Access®, Access 2, SYNCHRON LX®:
Access Wash Buffer II, Cat. No. A16792
UniCel® Dxi:
Unicel Dxi: Wash Buffer II, Cat. No. A16793

Provided ready to use. Stable until the expiration date stated on the label when stored at room temperature (15 to 30°C). An increase in substrate background measurements or increased relative light units for the zero calibrators in "sandwich"-type assays may indicate instability.

R3 Wash Buffer: TRIS buffered saline, surfactant, < 0.1 sodium azide, and 0.1% ProClin 300.

Refer to the appropriate system manuals and/or Help system for detailed instructions.
E. Quality Control (QC) materials: commercial control material.

F. Access Sample Diluent A
Cat. No. 81908: 4 mL/vial

The analyte level in patient samples may exceed the level of the specific S5 calibrator. If a quantitative value is required, it will be necessary to dilute the sample in order to determine the analyte concentration.

Provided ready to use. Allow the contents to stand for 10 minutes at room temperature. Mix gently by inverting before use. Avoid bubble formation. Stable until the expiration date stated on the vial label when stored at 2 to 10°C.

Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value of the specific assay. If a sample contains more analyte than the stated value of the S5 calibrator, dilute the sample following dilution instructions in the specific assay labeling under "Limitations of the Procedure" in the reagent pack section. Refer to the appropriate system manuals and/or Help system for instructions on how to enter a sample dilution in a test request.

Access Sample Diluent A: Buffered BSA matrix with surfactant, < 0.1% sodium azide, 0.5% ProClin 300.

G. Access Immunoassay System and supplies

H. Warnings and Precautions

1. For in vitro diagnostic use.

2. Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.

3. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up (13).

4. Xi. Irritant: 0.5% ProClin 300.
   R 43: May cause sensitization by skin contact.
   S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

5. Substrate is sensitive to air exposure. Keep tightly closed at all times. Do not pool bottles of substrate.

6. The Material Safety Data Sheet (MSDS) is available upon request.

**Calibration Details**

An active calibration curve is required for all tests. For the Access CK-MB assay, calibration is required every 56 days. Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

The Access CK-MB Calibrators are provided at six levels - zero and approximately 3, 10, 30, 100 and 300 ng/mL. Assay calibration data are valid up to 56 days.
Calibrators run in duplicate.

To order and load a calibration: From the DXI home screen choose SAMPLE MANAGER, then NEW REQUEST, then CALIBRATION. From the displayed list choose the appropriate Calibrator and lot. The screen will then display 2 sample racks and show how to load the calibrators: first rack holds S0, S1, S2, and S3; second rack holds S4 and S5. Enter the actual rack numbers you will use to hold calibrators, and when calibrators are loaded into cups on racks, load racks on to DXI to begin running the calibration.

Quality Control

Quality control materials simulate the characteristics of patient samples and are essential for monitoring the system performance of immunochemical assays. Because samples can be processed at any time in a "random access" format rather than a "batch" format, quality control materials should be included in each 24-hour time period (14). Include commercially available quality control materials that cover at least two levels of analyte. Follow manufacturer’s instructions for reconstitution and storage. Each laboratory should establish mean values and acceptable ranges to assure proper performance. Quality control results that do not fall within acceptable ranges may indicate invalid test results. Examine all test results generated since obtaining the last acceptable quality control test point for this analyte. Refer to the appropriate system manuals and/or Help system for information about reviewing quality control results.

Bio-Rad Cardiac Marker Plus Control LT, level 1 cat. 146, level 2 cat.147, and level 3 cat. 148. Controls are shipped and stored frozen at -25°C to -15°C. Once thawed (on rocker 30 minutes at room temp 18-25°C) and opened, vials are stable for 15 days when stored tightly capped at 2-8°C. Mix by gentle inversion prior to use.

Request QC samples by choosing SAMPLE MANAGER, NEW REQUEST, PATIENT/QC, enter rack ID, REQUEST QC, check the appropriate control, then choose the test "CK-MB."

Procedure

A. Access Instrument

Refer to the appropriate system manuals and/or Help system for preparation and operation.

B. Assay Procedure

Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.

Follow “DxI600 Sample and QC Analysis Guide” procedure for patient and QC sample analysis.

Results

A. Patient test results are determined automatically by the system software using a smoothing spline math model. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data. Patient test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

Reportable Range of assay is 1-300 ug/L. Results below the reportable range of assay are reported as <1 ug/L. Results above the reportable range of assay are reported as >300 ug/L.
B. Expected Values

1. Each laboratory should establish its own reference ranges to assure proper representation of specific populations.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>n</th>
<th>Median Age</th>
<th>Age Range</th>
<th>Reference Interval (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium heparin plasma and serum</td>
<td>242</td>
<td>48</td>
<td>23—78</td>
<td>0.6—6.3</td>
</tr>
<tr>
<td>EDTA plasma</td>
<td>242</td>
<td>48</td>
<td>23—78</td>
<td>0.5—5.0</td>
</tr>
</tbody>
</table>

2. A study performed by Beckman Coulter, Inc. on samples described below produced the following reference intervals. Reference intervals (95% central fraction) were generated based on observed sample type differences (i.e., heparin and serum vs. EDTA). Reference intervals were calculated using NCCLS C28-A2 guideline.

3. After acute myocardial infarction (AMI), CK-MB rises rapidly to peak levels within 12 hours, then declines to normal levels within 36–72 hours. The World Health Organization requires two of the following criteria for confirmation of AMI: evolutionary changes in the ECG, elevated cardiac enzymes and history of chest pain (5,6).
**Procedural Comments**

A. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.

B. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.

C. Use fifty-five (55) µL of sample for each determination in addition to the sample container and system dead volumes. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.

D. The system default unit of measure for sample results is ng/mL. To change sample reporting units to the International System of Units (SI units), µg/L, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply ng/mL by multiplication factor 1.

**Limitations of the Procedure**

A. Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value (approximately 0.1–300 ng/mL).

- If a sample contains less than the lower limit of detection for the assay, report the results as less than that value (i.e. < 0.1 ng/mL).

- If a sample contains more than the stated value of the highest Access CK-MB Calibrator (S5), report the result as greater than that value (i.e. > 300 ng/mL). Alternatively, dilute one volume of sample with equal volumes of Access Sample Diluent A. Refer to the appropriate system manuals and/or Help system for instructions on entering a sample dilution in a test request. The system reports the results adjusted for the dilution.

B. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples.(15,16)

Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.

C. The Access CK-MB results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests and other appropriate information.

D. Samples containing up to 10 mg/dL bilirubin, 3000 mg/dL Triglycerides (triglycerides), 500 mg/dL hemoglobin, or 6000 mg/dL of human serum albumin do not affect the concentration of CK-MB assayed. All CK-MB values obtained in the presence of each interferent were ± 10% of the control.

E. No significant cross-reactivity was observed when CK-BB (120 ng/mL) and CK-MM (35,000 ng/mL) were added to synthetic BSA matrix containing CK-MB.
F. The following drugs were added to normal human serum containing approximately 0.8 ng/mL CK-MB. Each drug was tested at a minimum concentration (listed below) of five times the therapeutic level. All CK-MB values obtained in the presence of each drug/interferent were ± 10% of the control. This study was based on NCCLS EP7-P guidelines.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration Tested (mg/dL)</th>
<th>Percent of Control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abciximab</td>
<td>2</td>
<td>101</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>20</td>
<td>98</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>40</td>
<td>96</td>
</tr>
<tr>
<td>Ambroxol</td>
<td>40</td>
<td>106</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>Ascorbic Acid</td>
<td>3</td>
<td>97</td>
</tr>
<tr>
<td>Aspirin</td>
<td>50</td>
<td>98</td>
</tr>
<tr>
<td>Atenolol</td>
<td>1</td>
<td>102</td>
</tr>
<tr>
<td>Caffeine</td>
<td>10</td>
<td>104</td>
</tr>
<tr>
<td>Captopril</td>
<td>5</td>
<td>103</td>
</tr>
<tr>
<td>Cinnarizine</td>
<td>40</td>
<td>102</td>
</tr>
<tr>
<td>Cocaine</td>
<td>5</td>
<td>105</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>2</td>
<td>102</td>
</tr>
<tr>
<td>Digoxin</td>
<td>0.02</td>
<td>96</td>
</tr>
<tr>
<td>Dopamine</td>
<td>65</td>
<td>101</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>Furosemide</td>
<td>40</td>
<td>92</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>40</td>
<td>100</td>
</tr>
<tr>
<td>Methyldopa</td>
<td>2.5</td>
<td>105</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>6</td>
<td>105</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>6.4</td>
<td>102</td>
</tr>
<tr>
<td>Nystatin</td>
<td>0.7</td>
<td>107</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>0.5</td>
<td>102</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>10</td>
<td>99</td>
</tr>
<tr>
<td>Propranolol</td>
<td>0.5</td>
<td>103</td>
</tr>
<tr>
<td>Quinidine</td>
<td>5</td>
<td>102</td>
</tr>
<tr>
<td>Theophylline</td>
<td>25</td>
<td>101</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>7.5</td>
<td>106</td>
</tr>
<tr>
<td>Verapamil</td>
<td>16</td>
<td>106</td>
</tr>
</tbody>
</table>

G. The lowest detectable level of CK-MB distinguishable from zero (Access CK-MB Calibrator S0) with 95% confidence is < 0.1 ng/mL (µg/L).

H. The Access CK-MB assay does not demonstrate any “hook” effect up to 20,000 ng/mL.

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**ProClin is a trademark of Rohm and Haas Company, or its subsidiaries or affiliates.
***Cosmocil CQ is a trademark of Avecia Ltd.

References

Beckman Coulter, Inc. Access CK-MB product insert, Fullerton, CA 92835, 387437.

Beckman Coulter, Inc. Access Substrate product insert, Fullerton, CA 92835, 386966.

Beckman Coulter, Inc. Access Wash Buffer II product insert, Fullerton, CA 92835, A16534 (Access), A16543 (UniCel Dxl).


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