DXI & ACCESS CK-MB

☒ St. Joseph Medical Center Tacoma, WA ☒ St. Clare Hospital Lakewood, WA ☒ St. Elizabeth Hospital Enumclaw, WA
☒ St. Francis Hospital Federal Way, WA ☒ St. Anthony Hospital Gig Harbor, WA ☐ Highline Medical Center Burien, WA ☐ PSC

PURPOSE

To provide instruction on how to perform CK-MB testing on the DXI & Access instruments.

PRINCIPLE

The CK-MB reagent, when used in conjunction with the Beckman Access or DXI Systems and Access Calibrators, is intended for quantitative determination of CK-MB concentration in human serum or plasma.

BACKGROUND

Clinical Significance

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. 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. 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.

Immuno-inhibition technology was originally used to measure CK-MB activitiy, which was compared with the measurement of total CK activity (ratio CK-MB/CK). 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.

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.

Methodology

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 sub-unit 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.

RELATED DOCUMENTS

R-PO-CH0810	Quality Control Program General Laboratory
R-PO-CH0809	Quality Control Westgard Rules Statistics
R-PR-AD0540	Specimen Rejection/Cancellation Protocol
J-F-CH0824	DXI & Access Controls
J-F-CH0825	DXI Calibrators
M-F-CH0820	Chemistry Controls
M-F-CH0826	Chemistry Calibrators
J-F-CH2000	Access 2 and DXI Analytical Measurement Range

SPECIMEN

Type of Specimen

Biological fluid samples should be collected in the same manner routinely used for any laboratory test. Freshly drawn serum or plasma is the preferred specimen.

Specimen Storage and Stability

1. Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum or plasma be physically separated from contact with cells within two hours from the time of collection.

2. Separated serum or plasma should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.

Sample Type	Volume	Sample Stability
Plasma/Serum	0.5ml	• 8 hours at 18-26° **C** • 48 hours at 2-8° **C** • After 48 hours, freeze at -15 to -20° C

Criteria for Unacceptable Specimens

Refer to the PROCEDURAL NOTES section of this chemistry information sheet for information on unacceptable specimens.
See Related Documents: Specimen Rejection/Cancellation Protocol.

Sample Volume

A filled 0.5 mL sample cup is the optimum volume. For optimum primary sample tube volumes in primary tube samples and minimum volumes, refer to the Primary Tube Sample Template for your system.

REAGENTS

1. 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.

Reactive Ingredients	
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.	R1a
Purified mouse IgG and purified goat IgG in buffered solution with BSA, 0.1% ProClin 300, and < 0.1% sodium azide	R1b
Mouse mononclonal anti-human CK-MB antibody alkaline phosphatase conjugate in buffered solution with BSA, 0.1% ProClin 300, and < 0.1% sodium azide.	R1c

2. 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 and or vial labels 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.

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

   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.

   Calibration Card: 1

3. 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.

Condition	Storage	Stability
Unopened	2 to 8°C	Until expiration date stated on the label
Equilibration prior to use (unopened)	15 to 30°C (room temperature)	Minimum 18 hours Maximum 14 days
In use (opened)	External fluids tray substrate position	Maximum 14 days

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.

4. **Access®, Access 2:**
   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.

5. Quality Control (QC) materials: commercial control material.

6. 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.

7. Access Sample Diluent A
   Cat. No. A79783: 2 diluent packs, 32.9 mL/pack. For use with the UniCel DxI system onboard dilution feature.

   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 samples in order to determine the analyte concentration.

   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 of each well. Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range. If the diluent pack is damaged (i.e., broken elastomer), discard the pack.

Samples can be accurately measured within the analytical 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.

R1a - R1e: Buffered BSA matrix with surfactant, < 0.1% sodium azide, 0.5% ProClin 300.

8. Access Immunoassay System and supplies

9. Warnings and Precautions
   - For *in vitro* diagnostic use.
   - 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.
   - 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).
   - 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.
   - Substrate is sensitive to air exposure. Keep tightly closed at all times. Do not pool bottles of substrate.
   - The Material Safety Data Sheet (MSDS) is available upon request.

CALIBRATION

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.

QUALITY CONTROL

See Related Documents J-F-CH0824 DXI & Access Controls and M-F-CH0820 Chemistry Controls

STEPS

1. Instrument Operation: Refer to the appropriate system manuals and/or Help system for preparation and operation.

2. 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.

RESULTS

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.

PERFORMANCE CHARACTERISTICS

Reference range

Type	Conventional Units
Serum or Plasma	0.0-9.0 ng/mL
Critical	> 9.0 ng/mL

For Critical Value reporting protocol, refer to FHS Critical Policy

Analytic Range

Type	Conventional Units
Serum or Plasma	0.1 -300 ng/mL

Reporting results outside the analytical range

Lower Limit of range	0.1 ng/mL	Results below 0.1 should be reported as <0.1 ng/mL
Upper limit of range	300 ng/mL	Results >300 should be diluted with Sample Diluent A, reanalyzed and dilution factor applied. The maximum allowable dilution is X2. Results >600 should be reported as >600 ng/mL.

LIMITATIONS

Substance	Level Tested	Observed Effect
Hemoglobin	500 mg/dL INDEX of 10	No significant interference
Bilirubin	10mg/dL INDEX of 7	No significant interference
Lipemia-Triolein (Triglycerides)	3000 mg/dL INDEX of 10	No significant interference

1. 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.

2. 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.

3. Samples containing up to 10 mg/dL bilirubin, 3000 mg/dL Triolein (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.

4. 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.

5. 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.

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

6. 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).

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

PROCEDURAL NOTES

1. 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.

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

3. 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.

4. 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.

REFERENCES

Beckman Coulter, Inc. Access CK-MB product insert, Fullerton, CA 92835, A34099.

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 DxI).

1. Tsung SH. Creatine kinase isoenzyme patterns in human tissue obtained at surgery. Clin Chem 1976; 22: 173-174.
2. Urdal P, Urdal K, Stromme JH. Cytoplasmic creatine kinase isoenzymes quantitated in tissue specimens obtained at surgery. Clin Chem 1983; 29: 310-313.
3. Goulle JP, Mechard D, Laine G, Jeanmet A, Cramer J, Maitrot B, Fondimare A, Gruchy D, Letac B. Répartition isozymique de la créatine kinase dans différents organes humains. Intérêt en pathologie humaine. Ann Biol Clin 1979; 37: 303-307.
4. El Allaf M, Chapelle JP, El Allaf D, Adam A, Faymonville ME, Laurent P, Heusghem C. Differentiating muscle damage from myocardial injury by means of the serum creatine kinase (CK) isoenzyme MB mass measurement/total CK activity ratio. Clin Chem 1986; 32: 291-295.
5. Apple F, Preese L. 1 Creatine Kinase-MB: Detection of myocardial infarction and monitoring reperfusion. Journal of Clinical Immunoassay 1994; 17(1): 24-29.
6. Adam JE, Abendschein DR, Jaffe AS. Biochemical markers of myocardial injury. Circulation 1993; 88(2): 750-763.
7. Galen RS, Reiffel JA, Gambino SR. Diagnosis of acute myocardial infarction: relative efficiency of serum enzyme and isoenzyme measurements. J Am Med Assoc 1975; 232: 145-147.
8. Mercer DW, Varat MA. Detection of cardiac specific creatine kinase isoenzyme in sera with normal or slightly increased total creatine-kinase activity. Clin Chem 1975; 21: 1088-1092.
9. Strom S, Bendz R. Serum creatine kinase (CK) activity after M-subunit inhibition in patients with atypical CK isoenzymes – a comparison to findings in acute myocardial infarction. Clin Chem Acta 1983; 132: 73-81.
10. Apple F, Preese L, Bennet R, Fredrickson A. Clinical and analytical evaluation of two immunoassays for direct measurement of creatine kinase MB with monoclonal anti-CK-MB antibodies. Clin Chem 1988; 4: 2364-2367.
11. Schwartz JG, Brown RW, McMahan CA, Gage CL, Herber SA. Clinical and analytical evaluation of different methods for measurement of creatine-kinase isoenzyme MB. Clin Chem 1989; 35: 130-134.
12. Approved Guideline – Procedures for the Handling and Processing of Blood Specimens, H18-A2. 1999. National Committee for Clinical Laboratory Standards.
13. DHHS (NIOSH) Publication No. 78-127, August 1976. Current Intelligence Bulletin 13 - Explosive Azide Hazard. Available http://www.cdc.gov/niosh.
14. Cembrowski GS, Carey RN. Laboratory quality management: QC & QA. ASCP Press, Chicago, IL, 1989.
15. Kricka L. Interferences in immunoassays – still a threat. Clin Chem 2000; 46: 1037.
16. Bjerner J, et al. Immunometric assay interference: incidence and prevention. Clin Chem 2002; 48: 613–621.

DOCUMENT APPROVAL Purpose of Document / Reason for Change:			
Standardized formatting using small tables. Added Maximum dilution. Incorporated Updated Index information.			
Committee Approval Date	☒ Date: 1/8/2015 ☐ NA – revision of department-specific document which is used at only one facility	**Medical Director Approval** *(Electronic Signature)*	Kaie Wilkinson, MD 8/25/15