TRAINING UPDATE

Lab Location:	SGMC & WAH	**Date Distributed:**	8/8/2016
Department:	Core	**Due Date:**	8/29/2016
		Implementation:	**8/29/2016**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Mass Creatine Kinase MB Isoenzyme (MMB) by Dimension Vista® System SGAH.C83 v1

Note: this has been converted to a system SOP

Description of change(s):

Section	Reason
Header	Add WAH
3.2	Specify anticoagulant
4,5,6	Remove labeling instructions and add general one
6.4, 6.5	Replace LIS with Unity Real Time
10.5	Move patient review from section 6
15	Update to new standard wording
16	Update document titles
17	Update PI revision dates

This revised SOP will be implemented on August 29, 2016

Document your compliance with this training update by taking the quiz in the MTS system.

Technical SOP

Title	**Mass Creatine Kinase MB Isoenzyme (MMB) by Dimension Vista® System**
Prepared by	Ashkan Chini Date: 6/22/2012
Owner	Robert SanLuis Date: 7/28/2016

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		

Review		
Print Name	**Signature**	**Date**

Form revised 2/02/2007

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Mass Creatine Kinase MB Isoenzyme	Dimension Vista® System	CKMB

Synonyms/Abbreviations
MMB, CKMB, Included in Batteries/Packages: CIEP4

Department
Chemistry

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2. ANALYTICAL PRINCIPLE

The MMB method is a homogeneous sandwich chemiluminescent immunoassay based on LOCI® technology. LOCI® reagents include two synthetic bead reagents and a biotinylated anti-mass creatine kinase MB isoenzyme monoclonal antibody fragment. The first bead reagent (Sensibeads) is coated with streptavidin and contains photosensitive dye. The second bead reagent (Chemibeads) is coated with a second anti-mass creatine kinase MB isoenzyme monoclonal antibody and contains chemiluminescent dye. Sample is incubated with Chemibeads and biotinylated antibody to form a bead-mass creatine kinase MB isoenzyme-biotinylated antibody sandwich. Sensibeads are added and bind to the biotin to form bead-pair immunocomplexes. Illumination of the complex by light at 680 nm generates singlet oxygen from Sensibeads which diffuses into the Chemibeads, triggering a chemiluminescent reaction. The resulting signal is measured at 612 nm and is a direct function of the mass creatine kinase MB isoenzyme concentration in the sample.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	Normal procedures for collecting and storing serum and plasma may be used for samples to be analyzed by this method.
Special Collection Procedures	N/A
Other	N/A

3.2 Specimen Type & Handling

Criteria		
Type	**-Preferred**	Plasma (Lithium Heparin)
	-Other Acceptable	Serum
Collection Container		Plasma: Mint green top tube (PST) Serum: Red top tube, Serum separator tube (SST)
Volume	**- Optimum**	1.0 mL
	- Minimum	0.5 mL
Transport Container and Temperature		Collection container or Plastic vial at room temperature
Stability & Storage Requirements		Room Temperature: 12 hours
		Refrigerated: 3 days
		Frozen: 1 month

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Criteria	
Timing Considerations	Serum or plasma should be physically separated from cells as soon as possible with a maximum limit of two hours from the time of collection.
Unacceptable Specimens & Actions to Take	Specimens that are unlabeled, improperly labeled, or those that do not meet the stated criteria are unacceptable. Request a recollection and credit the test with the appropriate LIS English text code for "test not performed" message. Examples: Quantity not sufficient-QNS; Wrong collection-UNAC. Document the request for recollection in the LIS.
Compromising Physical Characteristics	Gross hemolysis. Reject sample and request a recollection. Credit the test with the appropriate LIS English text code explanation of HMT (Specimen markedly hemolyzed)
Other Considerations	Allow Red Top or SST to clot completely prior to centrifugation.

NOTE: Labeling requirements for all reagents, calibrators and controls include: (1) Open date, (2) Substance name, (3) Lot number, (4) Date of preparation, (5) Expiration date, (6) Initials of tech, and (7) Any special storage instructions. Check all for visible signs of degradation. When placed onboard the analyzer, the instrument captures the date / time loaded and calculates and tracks the opened expiration.

4. REAGENTS

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included as a Related Document.
~~Refer to the Safety Data Sheet (SDS) supplied with the reagents for complete safety hazards.~~
~~Refer to the section in this procedure covering "SAFETY" for additional information.~~

4.1 Reagent Summary

Reagents	Supplier & Catalog Number
Mass Creatine Kinase MB Isoenzyme	Siemens, Flex® reagent cartridge, Cat. No. K6420

4.2 Reagent Preparation and Storage

Reagent	Mass Creatine Kinase MB Isoenzyme
Container	Reagent cartridge
Storage	Store at 2-8° C
Stability	• Reagent is stable until expiration date stamped on the reagent cartridges. • Sealed wells on the instrument are stable for 30 days.

	• Once wells 1 - 12 have been entered by the instrument, they are stable for 7 days.
Preparation	All reagents are liquid and ready to use.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
MMB CAL	Siemens Dimension Vista®, Cat. No. KC672

5.2 Calibrator Preparation and Storage

Calibrator	MMB CAL
Preparation	MMB CAL is ready for use. No preparation is required.
Storage/Stability	• Store at 2-8° C • **Unopened calibrator** is stable until expiration date stamped on the box. • **Opened Calibrator:** once the stopper of the vial is punctured, assigned values are stable for 7 days when stored on board the Dimension Vista System. • **Opened Calibrator:** once cap is removed, assigned values are stable for 30 days when recapped immediately after use and stored at 2-8° C. **Do not use this vial on board the instrument.**

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	MMB CAL
Assay Range	0.5 – 300.0 ng/mL
Suggested Calibration Level	See Reagent Package Insert for lot specific assigned values in ng/mL
Frequency	• Every new reagent cartridge lot. • Every 30 days for any one lot • When major maintenance is performed on the analyzer. • When control data indicates a significant shift in assay.
Calibration Scheme	6 levels, n = 3

5.4 Calibration Procedure

Auto Calibration:

1. Place the required calibrator vials in a carrier. Make sure the barcode labels are entirely visible through the slots.
2. Place the carrier in the loading area.
3. Position the carrier with the labels facing away from the user.
4. Press the **Load** button.
5. Automatic calibration requires that calibrators be on the instrument. As the time for processing approaches, the instrument reviews onboard inventory for the appropriate calibrators.

Manual Calibration:

1. Verify that calibrators and reagents are in inventory on the instrument.
2. Press **System > Method Summary > Calibration**.
3. Select a method from the sidebar menu. Press the **Order Calibration** button on the screen.
4. Verify that the information on the screen is correct. Verify that the calibrator lot is correct using the drop-down menu.
   a. When calibrating using Vials press **OK**.
   b. When calibrating using Cups, check the Use Cups box. This displays the rack and cup position fields. For additional cups use the positions in ascending order. Be sure to use the number of calibration levels and cups as specified in the method IFU. Scan the rack barcode and place calibrator cups in an adapter in position 1 on a rack. Press **OK** and load the rack on the instrument.
5. The status field in the calibration screen changes sequentially to Awaiting Scheduling, Preparing Calibrators and Processing.

5.5 Tolerance Limits

IF……	THEN……
If result fall within assay-specific specification, and QC values are within acceptable limits,	proceed with analysis
If result falls outside assay-specific specification, or QC values are out of Acceptable limits,	troubleshoot the assay and/or instrument and repeat calibration

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalog Number
Liquichek™ Cardiac Markers Plus Control Levels 1, 2 and 3	Bio-Rad Laboratories Cat # 181, 182 and 183

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6.2 Control Preparation and Storage

Control	Liquichek Cardiac Markers Plus Controls, Level 1, 2 and 3
Preparation	Allow the frozen control to thaw at room temperature (18-25°C) until completely thawed. Swirl the contents gently to ensure homogeneity. (Do not use a mechanical mixer) Use immediately. After each use, promptly replace the stopper and return to 2-8°C storage.
Storage/Stability	Unthawed controls are stable until the expiration date at -20 to -70° C. Once the control is thawed and opened, MMB will be stable for 20 days when stored tightly capped at 2-8°C.

6.3 Frequency

Analyze all levels of QC material after every calibration and each day of testing (notated on the QC frequency sheets posted on the instruments).

Refer to the Dimension Vista® QC Schedule in the Laboratory policy Quality Control Program and in the Dimension Vista® Quick Reference Guide.

6.4 Tolerance Limits and Criteria for Acceptable QC

Step	Action
1	Acceptable ranges for QC are programmed into the instrument's Quality Control software system and Unity Real Time, and may be posted near the instrument for use during computer downtime.
2	**Run Rejection Criteria** • Anytime the established parameters are exceeded (if one QC result exceeds 2 SD), the run is considered out of control (failed) and patient results must not be reported. • The technologist must follow the procedure in the Laboratory QC Program to resolve the problem.
3	**Corrective Action**: • All rejected runs must be effectively addressed through corrective action. Steps taken in response to QC failures must be documented. Patient samples in failed analytical runs must be reanalyzed according to the Laboratory QC Program. Supervisors may override rejection of partial or complete runs only with detailed documentation and criteria for overrides that are approved by the Medical Director. Consult corrective action guidelines in Laboratory QC Program. Follow corrective action guidelines in the Laboratory QC Program.

Step	Action
	• Corrective action documentation must follow the Laboratory Quality Control Program.
4	**Review of QC** • QC must be reviewed weekly by the Group Lead or designee and monthly by the Supervisor/Manager or designee. • If the SD and/or CV are greater than established ranges, investigate the cause for the imprecision and document implementation of corrective actions.

6.5 Documentation

- QC tolerance limits are programmed into the instrument and Unity Real Time; it calculates cumulative mean, SD and CV and stores all information for easy retrieval.
- Quality control records are reviewed daily at the bench, weekly by the Group Lead or designee, and monthly by the Supervisor/Manager or designee.
- Refer to complete policies and procedures for QC documentation and for record retention requirements in the Laboratory QC Program.

6.6 Quality Assurance Program

- Each new lot number of reagent or new shipment of the same lot of reagent must be tested with external control materials and previously analyzed samples. Performance of the new lot must be equivalent to the previous lot; utilize published TEA for acceptability criteria.
- Training must be successfully completed and documented prior to performing this test. This procedure must be incorporated into the departmental competency assessment program.
- The laboratory participates in CAP proficiency testing. All proficiency testing materials must be treated in the same manner as patient samples.
- Monthly QC must be presented to the Medical Director or designee for review and signature.
- Monthly QC mean and SD are sent to Bio-Rad Laboratories for peer group comparison.
- Consult the Laboratory QC Program for complete details.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

Dimension Vista® System

7.2 Equipment

- Refrigerator capable of sustaining 2–8°C.
- Freezer capable of sustaining range not to exceed -20 to -70°C.
- Centrifuge

7.3 Supplies

- Aliquot Plates
- System Fluids
- Assorted calibrated pipettes (MLA or equivalent) and disposable tips

8. PROCEDURE

MMB Flex® reagent cartridge Cat. No. K6420 is required to perform this test.

Mass Creatine Kinase MB Isoenzyme is performed on the Dimension Vista® System after the method is calibrated (see Reference Material in Calibration section) and Quality Controls are acceptable.

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

8.1	**Sample Processing**
1.	A sample rack holding tubes or cups is placed on the rack input lane.
2.	The sample shuttle moves the rack to the barcode reader which identifies the rack and samples to the system.
3.	The rack moves into the sample server and to the rack positioner.
4.	At the same time, aliquot plates move from the aliquot loader into position.
5.	The aliquot probe aspirates the sample from the tubes or cups and dispenses it into the wells of the aliquot plates.
6.	After each aspirate-dispense action, the probe is thoroughly rinsed inside and out to prevent sample carryover.
7.	When sample aspiration is completed, the sample server moves the rack back to the sample shuttle, where it is placed on the output lane and can be removed by the operator.

8.2	**Specimen Testing**
1.	For QC placement and frequency, refer to the Dimension Vista® QC Schedule in the Laboratory QC Program.
2.	Follow the instructions, outlined in the Dimension Vista® Operator's Manual

8.2	Specimen Testing
3.	The instrument reporting system contains error messages to warn the user of specific malfunctions. Results followed by such error messages should be held for follow-up. Refer to the Dimension Vista® system manual "Error messages" section for troubleshooting.
4.	Follow protocol in Section 10.5 "Repeat criteria and resulting" for samples with results above or below the Analytical Measurement Range (AMR). Investigate any failed delta result and repeat, if necessary.
5.	Append the appropriate English text code qualifier messages to any samples requiring a comment regarding sample quality and/or any other pertinent factors.

Test Conditions	
Sample Volume:	5 µL
Chemibead Reagent Volume:	20 µL
Biotinylated Antibody Volume:	20 µL
Sensibead Volume:	20 µL
Assay Buffer Volume:	60 µL
Reaction Time:	10 minutes
Test Temperature:	37° C
Wavelength:	680 & 612 nm
Type of measurement:	Chemiluminescence

NOTE: In the event that the test system becomes inoperable, notify supervision or designee for further direction. Patient specimens must be stored in a manner that maintains the integrity of the specimen.

9. CALCULATIONS

The instrument automatically calculates the concentration of MMB in ng/mL.

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

None required

10.2 Rounding

No rounding is necessary. Instrument reports results up to one decimal point.

10.3 Units of Measure

ng/mL

10.4 Clinically Reportable Range (CRR)

0.5 – 6,000.0 ng/mL

10.5 Review Patient Data

Each result is reviewed for error messages. Refer to the Dimension Vista system manual "Error messages" section for troubleshooting. Resolve any problems noted before issuing patient reports.

10.6 Repeat Criteria and Resulting

All repeats must replicate the original result within the total allowable error (TEa) of the assay. Refer to TEa listing for specific information.

Values that fall within the AMR or CRR may be reported without repeat. Values that fall outside these ranges must be repeated.

IF the result is …	THEN…
< 0.5 ng/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: < 0.5 ng/mL
≥ 300.0 ng/mL	**On Board Automated Dilution:** Results ≥ 300.0 ng/mL will automatically have repeat testing performed into the instrument using dilution factor of 20. No multiplication is necessary.
> 6,000.0 ng/mL	If the recommended dilution does not give results within the clinically reportable range, report as: "> 6,000.0 ng/mL-REP" Bring to the attention of your supervisor prior to releasing result.

Message	Code
Verified by repeat analysis	Append –REP to the result.

11. EXPECTED VALUES

11.1 Reference Ranges

0.0 – 3.6 ng/mL

11.2 Critical Values

None established

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

The creatine kinase MB isoenzyme (CKMB) is found primarily in cardiac tissue, with substantially lower concentrations also seen in skeletal muscle. The quantitation of CKMB is routinely ordered as part of the cardiac panel and is useful in the diagnosis of acute myocardial infarction (AMI). Typically, in cases of uncomplicated AMI, serial determinations show a pattern wherein CKMB levels become elevated within 4–8 hours after onset of pain, peak between 12–24 hours and then drop to normal by 48 hours. CKMB concentrations have also been used to assess the extent of AMI and subsequent reinfarction. Mass CKMB is the biochemical marker of choice for perioperative myocardial infarction during the first 48 hours after the onset of pain. The diagnostic sensitivity, specificity and efficiency of mass CKMB is superior to that of CK isoenzymes by electrophoresis.

13. PROCEDURE NOTES

- **FDA Status:** FDA Approved/cleared
- **Validated Test Modifications:** None

The instrument reporting system contains error messages to warn the operator of specific malfunctions. Any report slip containing such error messages should be held for follow-up. Refer to your Dimension Vista Operator's Guide.

The expected maximum observed standard deviations for repeatability using n = 5 replicates at the following MMB concentrations are:

MMB Concentration	Acceptable S.D. Maximum
5.5 ng/mL	1.3 ng/mL
91.6 ng/mL	7.9 ng/mL

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

0.5 – 300.0 ng/mL

14.2 Precision

Material	Mean ng/mL	Standard Deviation (%CV)	
		Repeatability	Within-Lab
Liquichek Cardiac Control			
Level 1	5.5	0.32 (5.8)	0.38 (6.8)
Level 2	91.6	1.89 (2.1)	3.24 (3.5)
Serum Pool	282.5	5.24 (1.9)	9.24 (3.3)

14.3 Interfering Substances

HIL Interference:
The MMB method was evaluated for interference according to CLSI/NCCLS EP7-A2. Bias, defined as the difference between the control sample (does not contain interferent) and the test sample (contains interferent), is shown in the table below. Bias exceeding 10% is considered "interference".

Substance tested	Substance Concentration	MMB ng/mL	Bias %
Hemoglobin (hemolysate)	500 mg/dL	5.7	<10
Bilirubin (unconjugated)	60 mg/dL	5.7	<10
Bilirubin (conjugated)	60 mg/dL	5.7	<10
Lipemia Intralipid®	3000 mg/dL	5.7	<10

14.4 Clinical Sensitivity/Specificity/Predictive Values

Not available

15. SAFETY

Refer to your local and corporate safety manuals and Safety Data Sheet (SDS) for detailed information on safety practices and procedures and a complete description of hazards.

16. RELATED DOCUMENTS

1. Dimension Vista® Clinical Chemistry System Operator's Manual
2. Dimension Vista® Calibration/Verification Procedure
3. Dimension Vista® Cal Accept Guidelines
4. Dimension Vista® Calibration summary
5. Dimension Vista® Sample Processing, Startup and Maintenance procedure
6. Laboratory Quality Control Program
7. QC Schedule for Siemens Dimension Vista®
8. Laboratory Safety Manual
9. Safety Data Sheets (SDS)
10. Dimension Vista® Limits Chart (AG.F200)
11. Quest Diagnostics Records Management Procedure
12. Dimension Vista® System Error Messages Chart
13. Centrifuge Use, Maintenance and Functions Checks (Lab policy)
14. Hemolysis, Icteria and Lipemia Interference (Lab policy)
15. Repeat Testing Requirement (Lab policy)
16. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
17. Current package insert MMB Flex® Reagent Cartridge 6420

17. REFERENCES

1. Package Insert, MMB Flex® Reagent Cartridge K6420, Siemens Healthcare Diagnostics Inc., 03/25/2015.
2. Package Insert, MMB CAL, Siemens Healthcare Diagnostics Inc., 03/2008.
3. Package Insert, Liquichek Cardiac Markers Plus Control, Bio-Rad Laboratories, 5/2014.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
000	8/1/16		Update owner	L Barrett	R SanLuis
000	8/1/16	Header	Add WAH	L Barrett	R SanLuis
000	8/1/16	3.2	Specify anticoagulant	L Barrett	R SanLuis
000	8/1/16	4,5,6	Remove labeling instructions and add general one	L Barrett	R SanLuis
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000	8/1/16	16	Update document titles	L Barrett	R SanLuis
000	8/1/16	17	Update package insert revision dates	L Barrett	R SanLuis
000	8/1/16	Footer	Version # leading zero's dropped due to new EDCS in use as of 10/7/13	L Barrett	R SanLuis

19. ADDENDA

None