TRAINING UPDATE

Lab Location:	GEC	**Date Distributed:**	8/15/2017
Department:	Core	**Due Date:**	8/31/2017
		Implementation:	**8/31/2017**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Cardiac Troponin-I by Dimension® Xpand Chemistry Analyzer GEC.C20 v5

Mass Creatine Kinase MB Isoenzyme by Dimension® Xpand Chemistry Analyzer GEC.C22 v3

Description of change(s):

Most changes are updates to the format

Section	Reason
4,5,6	Remove individual section labeling instructions and add general one
5.3	Remove specific calibration steps and reference separate SOP
10.5	Move patient review from section 6
10.6	Remove repeat value below AMR/CRR *
15	Update to new standard wording, move reagent hazard warning from 4.2
17	Update package insert dates

* This change will be made to Xpand assays as SOPs are revised

These revised SOPs will be implemented on August 31, 2017

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

Technical SOP

Title	**Cardiac Troponin-I by Dimension® Xpand Chemistry Analyzer**
Prepared by	Ashkan Chini Date: 3/24/2011
Owner	Robert SanLuis Date: 4/2/2015

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
Troponin-I	Dimension® Xpand Chemistry Analyzer	TROPI1

Synonyms/Abbreviations
Cardiac Troponin-I / TROP, TROPI, CTNI Troponin is part of battery/package CIEP4

Department
Chemistry

Form revised 2/02/2007

2. ANALYTICAL PRINCIPLE

The CTNI method is a one step enzyme immunoassay based on the "sandwich" principle. Sample is incubated with chromium dioxide particles coated with a monoclonal antibody specific for the cardiac troponin-I molecule, and a conjugate reagent [alkaline phosphatase (ALP)] labeled monoclonal antibody specific for cardiac troponin-I, to form a particle/cardiac troponin-I/conjugate sandwich. Unbound conjugate is removed by magnetic separation and washing. After separation and washing, the particle/cardiac troponin-I/conjugate sandwich is transferred to the cuvette where the sandwich bound ALP triggers an amplification cascade.* ALP dephosphorylates synthetic flavin adenine dinucleotide phosphate (FADP) to produce FAD. FAD binds to apo D-amino acid oxidase and converts it to active holo D-amino acid oxidase. Each molecule of holo D-amino acid oxidase then produces multiple molecules of hydrogen peroxide (H_2O_2) which, in the presence of horseradish peroxidase (HRP), convert 3,5-dichloro-2-hydroxybenzenesulfonic acid (DCHBS) and 4-aminoantipyrine (4-AAP) to a colored product that absorbs at 510 nm. The color change measured is directly proportional to the concentration of cardiac troponin-I present in the patient sample.

* Technology licensed from London Biotechnology, Ltd., London, U.K.

$$\text{cTnI} + \text{CrO}_2\text{-Ab} + \text{Fab'-ALP} \longrightarrow \text{CrO}_2\text{-Ab-cTnI-Fab'-ALP} + \text{CrO}_2\text{-Ab} + \text{Fab'-ALP}$$

$$\text{CrO}_2\text{-Ab-cTnI-Fab'-ALP} + \text{CrO}_2\text{-Ab} + \text{Fab'-ALP} \xrightarrow{\text{Wash}} \text{CrO}_2\text{-Ab-cTnI-Fab'-ALP} + \text{CrO}_2\text{-Ab}$$

(transferred to cuvette)

$$\text{FADP} \xrightarrow{\text{CrO}_2\text{-Ab-cTnI-Fab'-ALP}} \text{FAD} + \text{Pi}$$

$$\text{apo D-amino acid oxidase} \xrightarrow{\text{FAD}} \text{holo D-amino acid oxidase}$$

$$\text{D-proline} + \text{O}_2 \xrightarrow{\text{Holo D-amino acid oxidase}} \text{H}_2\text{O}_2$$

$$\text{H}_2\text{O}_2 + \text{DCHBS} + \text{4-AAP} \xrightarrow{\text{HRP}} \text{Color (absorbs at 510 nm)}$$

cTnI = cardiac troponin-I

Form revised 2/02/2007

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	Use normal procedures for blood collection. Collect anytime requested by physician. Serial samples are generally taken at 6-8 hour intervals over the first 48 hours after the onset of chest pain in patients suspected of suffering myocardial infarction.
Special Collection Procedures	N/A
Other	N/A

3.2 Specimen Type & Handling

Criteria		
Type -Preferred **-Other Acceptable**	Plasma (Lithium Heparin) Serum	
Collection Container	Plasma: Mint green top tube (PST) Serum: Red top tube, Serum separator tube (SST)	
Volume - Optimum **- Minimum**	1.0 mL 0.5 mL	
Transport Container and Temperature	Collection container or Plastic vial at room temperature	
Stability & Storage Requirements	Room Temperature:	(20-25°C) 8 hours
	Refrigerated:	(2-8°C) 2 days
	Frozen:	(-20°C or colder) 1 month
Timing Considerations	N/A	
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 redraw. Credit the test with the appropriate LIS English text code explanation of HMT (Specimen markedly hemolyzed)	
Other Considerations	Allow 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.

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.

4.1 Reagent Summary

Reagents	Supplier & Catalog Number
Cardiac Troponin-I	Siemens, Flex® reagent cartridge, Cat. No. RF421C

4.2 Reagent Preparation and Storage

Reagent	**Cardiac Troponin-I**
Container	Reagent cartridge
Storage	Store at 2-8° C
Stability	• Stable until expiration date stamped on reagent cartridges. • Sealed or unhydrated cartridge wells on the instrument are stable for 30 days. • Once wells 1 – 8 have been entered by the instrument, they are stable for 3 days.
Preparation	Hydrating, dilution and mixing are automatically performed by the instrument.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
Cardiac Troponin I Calibrator	Siemens Dimension®, Cat. No. RC421C

5.2 Calibrator Preparation and Storage

Calibrator	Cardiac Troponin I Calibrator
Preparation	Before use, thaw and equilibrate at room temperature for one hour (not to exceed two hours). Mix the contents of the vial by inverting gently ten (10) times. Do not use glass pipettes when transferring calibrators to sample cups.
Storage/Stability	• Store frozen at -25 to -15°C • **Unopened frozen vials:** stable until expiration date printed on the label. • **Unopened thawed vials:** stable for 5 days at 2-8°C. • **Opened thawed vials:** stable for 24 hours when thawed, recapped and stored at 2-8°C.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	Cardiac Troponin-I Calibrator
Assay Range	0.04 – 40.0 ng/mL
Suggested calibration level	See reagent package insert for lot specific assigned values in ng/mL
Frequency	• Every new reagent cartridge lot. • Every 60 days for any one lot. • When major maintenance is performed on the analyzer. • When control data indicates a significant shift in assay.
Calibration Scheme	Levels 1, 2 n = 4 Level 3 n = 3 Level 4, 5 n = 2
Assigned Coefficients	C_0 - 989.0 C_1 8439.0 C_2 - 2.9 C_3 101.0 C_4 0.5
Procedure	Refer to Calibration / Verification Siemens Dimension® Xpand procedure for specific instructions.

5.4 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

6.2 Control Preparation and Storage

Control	Liquichek Cardiac Markers Plus Control, Levels 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	**Thawed and opened**: Troponin I stable for 10 days at 2-8°C. **Unopened**: stable until the expiration date at -20 to -70°C.

6.3 Frequency

Analyze all levels of QC material after every calibration and each day of testing.

Refer to the Dimension Xpand® QC Schedule in the Laboratory policy Quality Control Program and in the Dimension X-pand® 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. • 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 Xpand® System

7.2 Equipment

- Refrigerator capable of sustaining 2–8°C.
- Freezer capable of sustaining range not to exceed -15 to -25°C.
- Centrifuge

7.3 Supplies

- Plastic serum tubes and serum cups
- Purified water (Millipore® or equivalent)
- Calibrated pipettes and disposable tips
- Reaction Vessels, Cat. No. RXV1A
- Chemistry Wash, Cat. No. RD701

- Reagent Probe Cleaner, Cat. No. RD702
- Sample Probe Cleaner, Cat. No. RD703

8. PROCEDURE

CTNI Flex® reagent cartridge Cat. No. RF421C is required to perform this test.

Troponin-I is performed on the Dimension Xpand® 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	Instrument Set-Up Protocol
1.	For instrument set up and operation: Refer to Startup and Maintenance, Siemens Dimension® Xpand procedure.
2.	Check reagent inventory
3.	Sampling, reagent delivery, mixing, processing, and printing of results are automatically performed by the Dimension® Xpand system. For details of the automated parameters, see below under "Test conditions."

8.2	Specimen/Reagent Preparation
1.	Centrifuge the specimens.
2.	Specimens are placed in Dimension® Xpand segments for analysis by the instrument. Refer to the Sample Processing, Siemens Dimension® Xpand procedure. The sample container (if not a primary tube) must contain sufficient quantity to accommodate the sample volume plus 50 µL of dead volume. Precise container filling is not required.

8.3	Specimen Testing
1.	For QC placement and frequency, refer to the Dimension® Xpand QC Schedule in the Laboratory QC Program.
2.	Follow the instructions, outlined in the Dimension® Xpand Operators Manual
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® Xpand 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). Repeat critical values and document according to Critical Values procedure. 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 Size:	50 µL	
Antibody-CrO_2:	25 µL	
Antibody-ALP:	40 µL	
Incubating Temp.:	42°C	
Incubation Period:	4.0 minutes	
Cuvette	**Reaction**	**Blanking**
Transfer Volume:	65 µL	0 µL
FADP Reagent Volume:	24 µL	24 µL
APO Reagent Volume:	24 µL	24 µL
Diluent Volume:	267 µL	332 µL
Temperature:	37.0°C	N/A
Reaction Time:	5.4 minutes	N/A
Wavelength:	510 and 700 nm	N/A
Type of Measurement:	Bichromatic rate	N/A

9. CALCULATIONS

The instrument automatically calculates and prints the concentration of Troponin-I 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 to two decimal points.

10.3 Units of Measure

ng/mL

10.4 Clinically Reportable Range (CRR)

0.04 - 200.00 ng/mL

10.5 Review Patient Data

Technologist must review each result with error messages. Refer to the Dimension Xpand® system manual “Error messages” section for troubleshooting. Check for unusual patterns, trends, or distributions in patient results (such as an unusually high

percentage of abnormal results). Resolve any problems noted before issuing patient reports.

10.6 Repeat Criteria and Resulting

Values that fall below or within the AMR or CRR may be reported without repeat.

Values that exceed the upper ranges must be repeated.
Any samples immediately following a sample that reached upper AMR and are above the upper normal limit will be repeated along with a low level control to ensure no carryover occurred.

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

Values requiring **manual dilution** must be repeated.

IF the result is …	THEN…
≤ 0.04 ng/mL	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: <0.04 ng/mL
≥ 40.00 ng/mL	**On Board Automated Dilution:** Results ≥ 40.00 ng/mL will automatically have repeat testing performed into the instrument using dilution factor of 2.5. No multiplication is necessary.
> 100.00 ng/mL	**Manual Dilution:** Using the primary tube, make the smallest dilution possible to bring the raw data within the AMR. Maximum allowable dilution: x 5 **Diluent:** Purified water. Enter dilution factor as a whole number on the "Enter Sample Data" screen. For values requiring manual dilution, report the assay with code of –REP
>200.00 ng/mL	If the recommended dilution does not give results within the clinically reportable range, report as: ">200.00 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

0.00 – 0.07 ng/mL

11.2 Critical Values

> 0.09 ng/mL

Treatment of **Subsequent critical values** for Troponin-I:
Only the first critical value must be called. Subsequent critical values for troponin must be documented by appending the code **TROPC** to the result. This code translates to "Laboratory value indicates a critical value previously reported."

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Troponin-I is the contractile regulatory protein complex of striated muscle. It is found periodically along the thin filament of the myofibrils, in conjunction with the protein tropomyosin. The troponin complex consists of three distinct polypeptide components: troponin-C (the calcium binding element), troponin-I (the actinomyosin ATPase inhibitory element), and troponin-T (the tropomyosin binding element). The complex serves to regulate the calcium-dependent interaction of myosin and actin and thus plays an integral role in muscle contraction.

Troponin-I exists in three distinct molecular forms which correspond to specific isotypes found in fast-twitch skeletal muscle, slow-twitch skeletal muscle, and heart, respectively.

Several reports in the literature have indicated that cardiac troponin-I is released into blood within hours of the onset of symptoms of myocardial infarction and that it remains elevated for several days post-infarction. The cumulative data from these reports indicate that troponin-I levels become abnormal 4-8 hours following onset of chest pain, peak at 12-16 hours, and remain elevated for 5-9 days following an infarction.

Measurement of cardiac troponin-I levels provide sensitive and specific determination of myocardial injury over a wide diagnostic window. Elevations in cardiac troponin-I levels have been observed across a spectrum of acute coronary syndromes including Q-wave MI, non-Q-wave MI and unstable angina. A significantly higher incidence of mortality has been observed in patients with non-Q-wave MI and unstable angina who have detectable levels of cardiac troponin-I. This suggests that cardiac troponin-I provides a means for risk stratification of these individuals.

Cardiac troponins are markers of myocardial necrosis and not just MI. Elevations of cardiac troponins in medical conditions other than MI have now been well described and these elevations reflect various levels of myocardial necrosis, outside of an ischemic context. These elevations should not be perceived as "false positives" and they should be taken into account due to the high prognostic value relative to morbidity and mortality. Conditions other

than MI that can cause increased troponin values include but are not limited to chest trauma, cardiac and non-cardiac surgery, congestive heart failure, renal failure, drug cardio-toxicity, inflammatory diseases such as myocarditis, pulmonary embolism, infiltrative diseases, and acute neurological disease. Although treatment should be based on the primary underlying condition, it is recognized that any troponin elevation is predictive of adverse outcomes, a fact that is increasingly considered during the medical decision process

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 Xpand Operator's Guide.

A system malfunction may exist if the following 5-test precision is observed:

Concentration	S.D.
2.0 ng/mL	> 0.20 ng/mL
25.0 ng/mL	> 1.50 ng/mL

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

0.04 – 40.00 ng/mL

14.2 Precision

Material	Mean ng/mL	Standard Deviation (%CV)	
		Within-run	Total
MAS Tru-Liquid Control			
Level 1	0.35	0.01 (2.7)	0.03 (7.7)
Level 2	5.28	0.05 (1.0)	0.22 (4.2)
Level 3	14.52	0.14 (1.0)	0.71 (4.9)
Serum Pool			
Level 1	0.08	0.01 (7.3)	0.01 (15.1)
Level 2	0.16	0.01 (4.0)	0.01 (9.2)
Level 3	0.47	0.01 (2.9)	0.03 (6.2)
Level 4	1.44	0.04 (2.6)	0.07 (5.2)
Level 5	27.71	0.53 (1.9)	0.99 (3.6)
Level 6	40.05	0.75 (1.9)	1.81 (4.5)

14.3 Interfering Substances

Patient samples may contain heterophile antibodies that could react in immunoassays to give falsely elevated or depressed results. This assay has been designed to minimize interference from heterophile antibodies. Complete elimination of the interference cannot be guaranteed. A test result that is inconsistent with the clinical picture and patient history should be interpreted with caution.

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.

Cardiac Troponin-I Flex® Reagent Cartridge and CTNI CAL may cause an allergic skin reaction. Flex contains 2-Chloracetamide. CAL contains 5-chloro-2-methyl-3(2h)-isothiazolone mixture with 2-methyl-3(2h)-isothiazolone. IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: Get medical advice/attention.

16. RELATED DOCUMENTS

1. Dimension Xpand® Clinical Chemistry System Operator's Manual
2. Calibration / Verification Siemens Dimension® Xpand procedure
3. Dimension Xpand® Cal Accept Guidelines
4. Dimension Xpand® Calibration summary
5. Sample Processing, Siemens Dimension® Xpand procedure
6. Start up and Maintenance, Siemens Dimension® Xpand procedure
7. Laboratory Quality Control Program
8. QC Schedule for Siemens Dimension Xpand®
9. Laboratory Safety Manual
10. Safety Data Sheets (SDS)
11. Siemens Dimension Xpand® Limits Chart (AG.F143)
12. Quest Diagnostics Records Management Procedure
13. Dimension Xpand® System Error Messages Chart
14. Centrifuge Use, Maintenance and Functions Checks (Lab policy)
15. Hemolysis, Icteria and Lipemia Interference (Lab policy)
16. Repeat Testing Requirements (Lab policy)
17. Critical Values (Lab policy)
18. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
19. Current package insert CTNI Flex® Reagent Cartridge RF421C

17. REFERENCES

1. Package Insert, CTNI Flex® Reagent Cartridge RF421C, Siemens Healthcare Diagnostics Inc., 3/9/2015.
2. Package insert, Cardiac Troponin-I Calibrator RC421C, Siemens Healthcare Diagnostics Inc., 3/2015.
3. Package insert, Liquichek Cardiac Markers Plus Control Levels 1, 2 & 3. Bio-Rad Laboratories, 8/2016.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes SOP C072.001		
000	7/15/11	6.7	Add use of published TEA for acceptability criteria	L Barrett	N Cacciabeve
000	7/15/11	10.5	Change repeat criteria to manual dilutions only	R SanLuis	N Cacciabeve
000	7/15/11	11.2	Requirement for subsequent critical values and interpretation of code revised	L Barrett	N Cacciabeve
000	7/15/11	15	Update to approved format	L Barrett	N Cacciabeve
001	2/8/12	5.3	Changed calibration level statement	A. Chini	J Buss
001	2/8/12	6.1 & 6.2	Updated QC information	A. Chini	J Buss
001	2/8/12	10.2	Correct rounding to 2 decimals	A. Chini	J Buss
001	2/8/12	10.5	Remove QNSR code	L Barrett	J Buss
001	2/8/12	10.5	Add repeat criteria for possible carryover	J Buss	J Buss
001	2/8/12	17	Updated References	A. Chini	J Buss
002	4/2/15		Update owner	L Barrett	R SanLuis
002	4/2/15	1, 7.1	Add analyzer name	L Barrett	R SanLuis
002	4/2/15	5.2	Change in frozen storage temperature	L Barrett	R SanLuis
002	4/2/15	6.2	Update stability to 10 days	L Barrett	R SanLuis
002	4/2/15	6.4, 6.6	Replace LIS with Unity Real Time	L Barrett	R SanLuis
002	4/2/15	7.2	Change freezer requirements	L Barrett	R SanLuis
002	4/2/15	8.2	Remove Lynx, specify Xpand process	L Barrett	R SanLuis
002	4/2/15	Footer	Version # leading zero's dropped due to new EDCS in use as of 10/7/13	L Barrett	R SanLuis
3	7/6/15	3.2	Specify anticoagulant	L Barrett	R SanLuis
3	7/6/15	11.1	Change upper value from 0.10 to 0.07	L Barrett	R SanLuis
3	7/6/15	11.2	Change critical from ≥0.60 to >0.09	L Barrett	R SanLuis
4	7/26/17	4,5,6	Remove individual section labeling instructions and add general one	L Barrett	R SanLuis

Version	Date	Section	Reason	Reviser	Approval
4	7/26/17	5.3	Remove specific calibration steps and reference separate SOP	L Barrett	R SanLuis
4	7/26/17	10.5	Move patient review from section 6	L Barrett	R SanLuis
4	7/26/17	10.6	Remove repeat value below AMR/CRR	L Barrett	R SanLuis
4	7/26/17	15	Update to new standard wording, move reagent hazard warning from 4.2	L Barrett	R SanLuis
4	7/26/17	17	Update package insert dates	L Barrett	R SanLuis

19. ADDENDA

None

Technical SOP

Title	**Mass Creatine Kinase MB Isoenzyme by Dimension® Xpand Chemistry Analyzer**
Prepared by	Ashkan Chini Date: 3/25/2011
Owner	Robert SanLuis Date: 6/8/2015

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® Xpand Chemistry Analyzer	CKMB

Synonyms/Abbreviations
CK-MB, MMB, Part of battery/ package: CIEP4 (Cardiac Isoenzyme Profile)

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The MMB method is a one-step enzyme immunoassay based on the "sandwich" principle. The sample is incubated with chromium dioxide particles coated with monoclonal antibodies specific for CKB subunit, and conjugate reagent (ß-galactosidase labeled monoclonal antibodies specific for CKMB isoenzyme). A particle/CKMB/conjugate sandwich forms during the incubation period. Unbound conjugate is removed by magnetic separation and washing. The sandwich bound b-galactosidase is combined with a chromogenic substrate chlorophenol red-ß-d-galactopyranoside (CPRG). Hydrolysis of CPRG releases a chromophore (CPR). The concentration of CKMB present in the patient sample is directly proportional to the rate of color change due to formation of CPR measured at 577 nm. The amount of CKMB protein is measured immunologically and the results are reported in mass units (ng/mL or µg/L).

CKMB + $Cr0_2$-Ab + F(ab') $_2$-ß-galactosidase ⟶ $Cr0_2$-Ab-CKMB-F(ab')$_2$- ß-gal +CrO2-Ab + F(ab')$_2$-ß-galactosidase

$Cr0_2$-Ab-CKMB-F(ab') $_2$-ß-gal +$Cr0_2$-Ab + F(ab')$_2$-ß-galactosidase ⟶ (Wash) $Cr0_2$-Ab-CKMB-F(ab')$_2$-ß-gal +$Cr0_2$-Ab (transferred to cuvette)

CPRG (non-absorbing at 577nm) ⟶ ($Cr0_2$-Ab-CKMB-F(ab')$_2$-ß-gal) CPR (absorbs at 577 nm)

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	None
Other	N/A

3.2 Specimen Type & Handling

Criteria	
Type -Preferred **-Other Acceptable**	Plasma (Lithium Heparin) Serum
Collection Container	Plasma: Mint green top tube (PST) Serum: Red top tube, Serum separator tube (SST)
Volume - Optimum **- Minimum**	1.0 mL 0.5 mL
Transport Container and Temperature	Collection tube or plastic vial at room temperature
Stability & Storage Requirements	Room Temperature: 12 hours
	Refrigerated: 3 days
	Frozen: (-20°C or colder) 1 month
Timing Considerations	N/A
Unacceptable Specimens & Actions to Take	Specimens that are unlabeled, improperly labeled, or those that do not meet the stated criteria are unacceptable. Blood collection tubes containing oxalate should not be used. 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.
Other Considerations	Allow to clot completely prior to centrifugation. Specimens should be free of particulate matter.

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.

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.

4.1 Reagent Summary

Reagents	Supplier & Catalog Number
Mass Creatine Kinase MB Isoenzyme	Siemens, Flex® reagent cartridge, Cat. No. RF420
Sample Diluent	Dimension® clinical chemistry system, REF791092901

4.2 Reagent Preparation and Storage

Reagent	**Mass Creatine Kinase MB Isoenzyme**
Container	Reagent cartridge
Storage	Store at 2-8°C
Stability	• Stable until expiration date stamped on the cartridges. • Sealed or unhydrated cartridge wells on the instrument are stable for 30 days. • Open well stability - o 3 days for wells 1 – 6 o 10 days for wells 7 and 8
Preparation	Reagents are supplied ready for use. No additional preparation is required.

Reagent	**Sample Diluent**
Container	Manufacturer supplied vial
Storage	Store at 2-8°C
Stability	Opened or unopened: stable until xpiration date stamped on the vial.
Preparation	Ready for use. No preparation is required.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
Mass Creatine Kinase MB Isoenzyme Calibrator	Siemens Dimension®, Cat. No. RC420

5.2 Calibrator Preparation and Storage

Calibrator	Mass Creatine Kinase MB Isoenzyme Calibrator
Preparation	1. Remove stopper and volumetrically add 2.00 ± 0.02 mL Reagent Grade Water. The water should be equilibrated to room temperature. 2. Replace stopper, invert gently 10 times and swirl gently for 10 seconds. 3. Let vials stand on bench top or gently mix for 15-20 minutes until the cake is completely dissolved. 4. Use immediately or refrigerate at 2-8°C for future use. Swirl gently prior to use.

Storage/Stability	• Store at 2-8°C before and after reconstitution. • Unopened: Stable until the label expiration date. • Once opened, stable for 24 hours after reconstitution when stoppered and stored at 2-8°C.

5.3 Calibration Parameter

Criteria	Special Notations
Reference Material	Mass Creatine Kinase MB Isoenzyme Calibrator
Assay Range	0.5 – 300 ng/mL
Calibration levels	See reagent package insert for lot specific assigned values in ng/mL
Frequency	• Every new reagent cartridge lot. • Every 60 days for any one lot • When major maintenance is performed on the analyzer. • When control data indicates a significant shift in assay.
Calibration Scheme	Levels 1, 2 n = 3 Level 3 n = 2 Level 4 n = 3 Level 5 n = 2
Assigned Coefficients	C_0 - 130 C_1 1800 C_2 - 3.7 C_3 750 C_4 0.5
Procedure	Refer to Calibration / Verification Siemens Dimension® Xpand procedure for specific instructions.

5.4 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

6.2 Control Preparation and Storage

Control	Liquichek Cardiac Markers Plus Control, Levels 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	**Thawed and opened**: CKMB stable for 20 days at 2-8°C. **Unopened**: stable until the expiration date at -20 to -70°C.

6.3 Frequency

Analyze all levels of QC material after every calibration and each day of testing.

Refer to the Dimension Xpand® QC Schedule in the Laboratory policy Quality Control Program and in the Dimension Xpand® 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. • Corrective action documentation must follow the Laboratory Quality Control Program.

Step	Action
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 Xpand® 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

- Plastic serum tubes and serum cups
- Millipore® water
- Calibrated pipettes and disposable tips
- Reaction Vessels, Cat. No. RXV1A
- Chemistry Wash, Cat. No. RD701

8. PROCEDURE

MMB Flex® reagent cartridge Cat. No. RF420 is required to perform this test.

Mass Creatine Kinase MB Isoenzyme is performed on the Dimension Xpand® 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	Instrument Set-Up Protocol
1.	For instrument set up and operation: Refer to Startup and Maintenance, Siemens Dimension® Xpand procedure.
2.	Check reagent inventory
3.	Sampling, reagent delivery, mixing, processing, and printing of results are automatically performed by the Dimension® Xpand system. For details of the automated parameters, see below under “Test conditions.”

8.2	Specimen/Reagent Preparation
1.	Centrifuge the specimens.
2.	Specimens are placed in Dimension® Xpand segments for analysis by the instrument. Refer to the Sample Processing, Siemens Dimension® Xpand procedure. The sample container (if not a primary tube) must contain sufficient quantity to accommodate the sample volume plus 50 µL of dead volume. Precise container filling is not required.

8.3	Specimen Testing
1.	For QC placement and frequency, refer to the Dimension® Xpand QC Schedule in the Laboratory QC Program.
2.	Follow the instructions, outlined in the Dimension® Xpand Operators Manual
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® Xpand system manual “Error messages” section for troubleshooting.

8.3	Specimen Testing
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	
Reaction Vessel	
Sample Size:	60 µL
Antibody-CrO_2:	50 µL
MMB Ab-ß-galactosidase:	50 µL
Incubation Temp:	42°C
Incubation Period:	8 minutes
Wash Steps:	5 minutes
Cuvette	
Transfer Volume:	40 µL
Substrate Reagent Volume (CRPG):	91 µL
Diluent Volume:	279 µL
Temperature:	37°C
Incubation Period:	4 minutes
Wavelength:	577 and 700 nm
Type of Measurement:	Bichromatic rate

9. CALCULATIONS

The instrument automatically calculates and prints the concentration of Mass Creatine Kinase MB Isoenzyme 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 to one decimal place.

10.3 Units of Measure

ng/mL

10.4 Clinically Reportable Range (CRR)

0.5 – 1500.0 ng/mL

10.5 Review Patient Data

Technologist must review each result with error messages. Refer to the Dimension Xpand® system manual "Error messages" section for troubleshooting. Check for unusual patterns, trends, or distributions in patient results (such as an unusually high percentage of abnormal results). 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 below or within the AMR or CRR may be reported without repeat. Values that exceed the upper 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 2. No multiplication is necessary.
>600.0 ng/mL	**Manual Dilution:** Using the primary tube, make the smallest dilution possible to bring the raw data within the AMR. Maximum allowable dilution: x 5 **Diluent**: Sample diluent Enter dilution factor as a whole number on the "Enter Sample Data" screen.
>1500.0 ng/mL	If the recommended dilution does not give results within the clinically reportable range, report as: ">1500.0 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. Mass CKMB is the biochemical marker of choice for perioperative myocardial infarction during the first 48 hours after the onset of pain. CKMB concentrations have also been used to assess the extent of AMI and subsequent reinfarction. 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 Xpand Operator's Guide.

A system malfunction may exist if the following 5-test precision is observed:

Concentration	**S.D.**
0 ng/mL	> 1.0 ng/mL
10 ng/mL	> 1.1 ng/mL
300 ng/mL	> 20.0 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)	
		Within-run	Between-day
Serum Pool			
Level 1	1.27	0.29	0.29
Level 2	7.76	0.27	0.69
Level 3	13.07	0.32	0.57
Dade CK-MB/Myoglobin Immunoassay Control			
Level 1	3.58	0.24	0.35
Level 2	16.69	0.43	1.10
Level 3	46.05	0.64	1.86

14.3 Interfering Substances

No clinically significant interference was observed form icterus (bilrubin 60 mg/dL), hemolysis (hemoglobin 1000 mg/dL) or lipemia (triglyceride 1500 mg/dL).

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.

MMB Flex® Reagent Cartridge and Sample Diluent may cause an allergic skin reaction. Flex contains 5-chloro-2-methyl-3(2h)-isothiazolone mixture with 2-methyl-3(2h)-isothiazolone. Diluent contains 2-Chloracetamide. IF ON SKIN: Wash with plenty of soap and water. If skin irritation or rash occurs: Get medical advice/attention.

16. RELATED DOCUMENTS

1. Dimension Xpand® Clinical Chemistry System Operator's Manual
2. Calibration / Verification Siemens Dimension® Xpand procedure
3. Dimension Xpand® Cal Accept Guidelines
4. Dimension Xpand® Calibration summary
5. Sample Processing, Siemens Dimension® Xpand procedure
6. Start up and Maintenance, Siemens Dimension® Xpand procedure
7. Laboratory Quality Control Program
8. QC Schedule for Siemens Dimension Xpand®
9. Laboratory Safety Manual
10. Safety Data Sheets (SDS)
11. Siemens Dimension Xpand® Limits Chart (AG.F143)

12. Quest Diagnostics Records Management Procedure
13. Dimension Xpand® System Error Messages Chart
14. Centrifuge Use, Maintenance and Functions Checks (Lab policy)
15. Hemolysis, Icteria and Lipemia Interference (Lab policy)
16. Repeat Testing Requirements (Lab policy)
17. Current Allowable Total Error Specifications at http://questnet1.qdx.com/Business_Groups/Medical/qc/docs/qc_bpt_tea.xls
18. Current package insert MMB Flex® Reagent Cartridge RF420

17. REFERENCES

1. Package Insert, MMB Flex® Reagent Cartridge RF420, Siemens Healthcare Diagnostics Inc., 3/25/2015.
2. Package Insert, Mass Creatine Kinase MB Isoenzyme Calibrator RC420, Siemens Healthcare Diagnostics Inc., 4/2016.
3. Package Insert, Liquichek Cardiac Markers Plus Control Levels 1, 2 & 3, Bio-Rad Laboratories, 8/2016.
4. Package Insert, Sample diluent REF791092901, 5/2015.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes SOP C073.001		
000	2/9/12		Update owner	L Barrett	J Buss
000	2/9/12	3.2	Edited temperatures	A Chini	J Buss
000	2/9/12	5.3	Edited Calibration Level statement	A Chini	J Buss
000	2/9/12	6.1 & 6.2	Updated QC information	A Chini	J Buss
000	2/9/12	6.7	Add use of TEA for lot to lot runs	L Barrett	J Buss
000	2/9/12	10.5	Remove QNSR code	L Barrett	J Buss
000	2/9/12	15	Update to standard wording	L Barrett	J Buss
000	2/9/12	17	Updated References	A Chini	J Buss
001	6/8/15		Update owner	L Barrett	R SanLuis
001	6/8/15	1, 7.1	Add analyzer name	L Barrett	R SanLuis
001	6/8/15	3.2	Specify anticoagulant	L Barrett	R SanLuis
001	6/8/15	4.2	Add hazard statement for diluent	L Barrett	R SanLuis
001	6/8/15	6.4,6.6	Replace LIS with Unity Real Time	L Barrett	R SanLuis
001	6/8/15	8.2	Remove Lynx	L Barrett	R SanLuis
001	6/8/15	10.5	Remove use of code REP from dilutions	A Chini	R SanLuis
001	6/8/15	16	Update document titles	L Barrett	R SanLuis
001	6/8/15	Footer	Version # leading zero's dropped due to new EDCS in use as of 10/7/13	L Barrett	R SanLuis

Version	Date	Section	Reason	Reviser	Approval
2	8/2/17	4,5,6	Remove individual section labeling instructions and add general one	L Barrett	R SanLuis
2	8/2/17	5.3	Remove specific calibration steps and reference separate SOP	L Barrett	R SanLuis
2	8/2/17	10.5	Move patient review from section 6	L Barrett	R SanLuis
2	8/2/17	10.6	Remove repeat value below AMR/CRR	L Barrett	R SanLuis
2	8/2/17	15	Update to new standard wording, move reagent hazard warning from 4.2	L Barrett	R SanLuis
2	8/2/17	17	Update package insert dates	L Barrett	R SanLuis

19. ADDENDA

None