Quest Diagnostics

TRAINING UPDATE

Lab Location:	GEC, SGAH & WAH	**Date Distributed:**	7/3/2012
Department:	Core	**Due Date:**	7/31/2012

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Creatine Kinase by Dimension® Chemistry Analyzer GEC.C21, SGAH.C51,WAH.C50 v002

Description of change(s):

Section	Reason
10.4	Correct CRR lower limit

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

Approved draft for training all sites (version 002)

Technical SOP

Title	**Creatine Kinase by Dimension® Chemistry Analyzer**
Prepared by	Ashkan Chini Date: 3/30/2011
Owner	Robert SanLuis, Jean Buss Date: 3/30/2011

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

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

Form revised 3/31/00

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Creatine Kinase	Dimension® Chemistry Analyzer	CPK

Synonyms/Abbreviations
CK, CPK, CKI

Department
Chemistry

Form revised 3/31/00

2. ANALYTICAL PRINCIPLE

CK catalyzes the transphosphorylation of phosphate from creatine phosphate to adenosine-diphosphate (ADP) producing adenosine-triphosphate (ATP). Hexokinase (HK) phosphorylates glucose from the ATP to phosphorylate glucose. The resulting glucose-6-phosphate is oxidized by glucose-6-phosphate dehydrogenase (G-6-PDH) with the simultaneous reduction of nicotinamide adenine dinucleotide phosphate (NADP). The rate of formation of NADPH is directly proportional to the CK activity in the sample and is measured bichromatically at 340 and 540 nm.

$$\text{Creatine phosphate + ADP} \xrightarrow[Mg^{++},\ NAC,\ EDTA]{CK} \text{Creatine + ATP}$$

$$\text{ATP + Glucose} \xrightarrow[Mg^{++}]{HK} \text{ADP + Glucose-6-phosphate}$$

$$\text{Glucose-6-phosphate} + NADP^{+} \xrightarrow{G\text{-}6\text{-}PDH} \text{6-Phosphogluconolactone + NADPH + } (H^{+})$$

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 (Heparin) Serum
Collection Container	Plasma: Green top tube Serum: Red top tube, Serum separator tube (SST)

Criteria	
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: 2 hours
	Refrigerated: (2-8°C) 7 days
	Frozen: (-20°C or colder) 1 month
Timing Considerations	Serum 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 to clot completely prior to centrifugation.

4. REAGENTS

Refer to the Material Safety Data Sheet (MSDS) 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	Quantity
Creatine Kinase	Siemens, Flex® reagent cartridge, Cat. No. DF38	4 Flex/carton
Enzyme Diluent	Dimension® clinical chemistry system REF 790035901	10 per box

4.2 Reagent Preparation and Storage

NOTES: Date and initial all reagents upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, (6) any special storage instructions; check for visible signs of degradation.
Refer to the Material Safety Data Sheet (MSDS) for a complete description of hazards. If a specific hazard is present, it will be noted in this procedure when the hazard is first encountered in a procedural step.

Form revised 3/31/00

Irritant. May cause sensitization by skin contact.

Reagent	**Creatine Kinase**
Container	Reagent cartridge
Storage	Store at 2-8°C
Stability	• Reagent is stable until expiration date stamped on the reagent cartridges. • Sealed or unhydrated cartridge wells on the instrument are stable for 30 days. • Once wells 1 – 4 have been entered by the instrument, they are stable for 5 days. • Once wells 5 – 6 have been entered by the instrument, they are stable for 10 days.
Preparation	Reagents are supplied ready for use. No additional preparation is required.

Reagent	**Enzyme Diluent**
Container	Manufacturer supplied vial
Storage	Store at 2-8° C before and after reconstitution
Stability	• Unreconstituted product is stable until expiration date stamped on the vial. • Reconstituted product is stable for 7 days following reconstitution or immediately if visible turbidity appears.
Preparation	• Reconstitute with exactly 10 mL of purified water. The water should be equilibrated to room temperature (22-28° C). • Replace stopper and invert gently 10 times. • Let vials stand on bench top for 15 minutes then invert gently 10 times. • Let vials stand on bench top an additional 15 minutes. • Then invert 10 times and swirl gently. • Use immediately or refrigerate at 2-8°C. • Before use, allow product to come to room temperature, then invert 10 times and swirl gently.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
Creatine Kinase Verifier	Siemens Dimension®, Cat. No. DC26

5.2 Calibrator Preparation and Storage

NOTE: Date and initial all calibrators upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech (6) any special storage instructions; check for visible signs of degradation.

Calibrator	Creatine Kinase Verifier
Preparation	• Remove vials from refrigerator and allow to stand at room temperature (22-28°C) for 10 to 15 minutes. • Volumetrically add 1.00 ± 0.01 mL of Millipore® water to each vial. • Replace stopper, and let stand for 5 minutes. Do not invert. • Swirl vials gently for 30 seconds, and then gently invert 10 times. • Let vials stand for 10 minutes, and then gently invert 10 times. • Let vial stand for additional 15 minutes. Then invert 10 times and swirl gently. • Use immediately or refrigerate at 2-8°C for future use. Prior to use invert 10 times and swirl gently.
Storage/Stability	• Store at 2-8°C. • The unopened reagents are stable until the expiration date printed on the label. • Reconstituted reagents are stable for 8 hours when vials are stoppered and stored at 2-8°C.

5.3 Calibration Parameter

Criteria	**Special Notations**
Reference Material	Creatine Kinase Verifier
Assay Range	7 – 1000 U/L
Suggested calibration level	0, 525, 1100 U/L
Frequency	• Every new reagent cartridge lot. • Every 90 days for any one lot. • When major maintenance is performed on the analyzer. • When control data indicates a significant shift in assay.
Calibration Scheme	Three levels in triplicate.
Assigned Coefficients	C_0 1.708 C_1 8.044

5.4 Calibration Procedure

1. From Operating Menu press F5:Process Control press F1: Calibration Enter Password press F2: SETUP and RUN
2. Select the test method to be calibrated - if lot number is incorrect Press F1: Other Lot
3. Enter all information on screen
4. Press F8: QC yes/no to change to yes
5. Press F4: Assign cups If additional methods need to be calibrated, select the method.
6. Press F7: Load/run
7. Load cups into assigned position
8. Press F4: RUN

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™ Unassayed Chemistry Controls Levels 1 & 2	Bio-Rad Laboratories Catalog # 691 & 692

6.2 Control Preparation and Storage

NOTE: Date and initial all controls upon opening. Each container should be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, and (6) any special storage instructions; check for visible signs of degradation.

Control	Liquichek Unassayed Chemistry Controls Levels 1 & 2

Preparation	Allow the frozen control to stand 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	Open controls are stable for 15 days at 2-8°C. Unopened controls are 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 (notated on the QC frequency sheets posted on the instruments).

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

6.4 Tolerance Limits

Step	Action
1	Acceptable ranges for QC are programmed into the instrument's Quality Control software system, into the Laboratory Information System (LIS), 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.

Form revised 3/31/00

Step	Action
	• 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 Review Patient Data

Technologist must review each result print-out for error messages. Refer to the Dimension® 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.

6.6 Documentation

- QC tolerance limits are programmed into the instrument and the LIS. The LIS 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 Lead Technologist 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.

6.7 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.
- 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 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® Chemistry Analyzer

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
- Purified water (Millipore® or equivalent)
- Calibrated pipettes and disposable tips

8. PROCEDURE

CKI Flex® reagent cartridge Cat. No. DF38 is required to perform this test.

Creatine Kinase is performed on the Dimension® clinical chemistry 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.

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.

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

8.2	**Specimen/Reagent Preparation**
1.	Centrifuge the specimens.
2.	For sites with StreamLab (Lynx): **Qualifying** samples are loaded via the Dimension® Lynx System which automatically routes specimens to the instruments. Refer to the Dimension® Streamlab® Analytical Workcell (Lynx) System manual for instructions.
3.	Alternatively, specimens are placed in color-coded Dimension® segments for analysis by the instrument. Refer to the Sample Processing, Siemens Dimension® 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® QC Schedule in the Laboratory QC Program.

Form revised 3/31/00

8.3	Specimen Testing
2.	Follow the instructions, outlined in the Dimension® 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® 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 Size:	14 µL
Reagent 1 Volume:	112 µL
Reagent 2 Volume:	55 µL
Reaction Time:	8.7 minutes
Temperature:	37° C
Wavelength:	340 and 540 nm
Type of Measurement:	Bichromatic rate

9. CALCULATIONS

The instrument automatically calculates and prints the concentration of Creatine Kinase in U/L.

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

None required

10.2 Rounding

No rounding is necessary. Instrument reports results as a whole number.

10.3 Units of Measure

U/L

10.4 Clinically Reportable Range (CRR)

7 – 20,000 U/L

10.5 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…
<7 U/L	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: <7 U/L
≥1,000 U/L	**On Board Automated Dilution:** Results ≥1,000 U/L will automatically have repeat testing performed into the instrument using dilution factor of 7. No multiplication is necessary. Append the result with code –REP.
>7,000 U/L	**Manual Dilution**: Using the primary tube, make the smallest dilution possible to bring the raw data within the AMR. Maximum allowable dilution: x 20 **Diluent**: Enzyme diluent. Enter dilution factor as a whole number on the "Enter Sample Data" screen. Report the assay with code of –REP.
>20,000 U/L	If the recommended dilution does not give results within the clinically reportable range, report as: ">20,000 U/L-REP" Bring to the attention of your supervisor prior to releasing result.

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

11. EXPECTED VALUES

11.1 Reference Ranges

Age	Female	Male
Adult (>18 years):	21 – 215 U/L	32 – 232 U/L
Pediatric:		
0– 90 days	43-474	29-303
3 – 12 months	27-242	25-172
13 months – 23 months	25-177	28-162
2 – 10 years	25-177	31-152
11 – 14 years	31-172	31-152
15 – 18 years	28-142	34-147

11.2 Critical Values

None established

11.3 Priority 3 Limit(s)

None established

12. CLINICAL SIGNIFICANCE

Measurements of Creatine Kinase are used in the diagnosis and treatment of myocardial infarction and muscle disease, such as progressive Duchenne-type muscular dystrophy. Creatine Kinase (CK) is an enzyme that is found primarily in skeletal muscle, cardiac muscle and brain tissue. Elevated levels of CK are associated with myocardial infarction and various muscle disorders. In myocardial infarction, peak CK levels occur 24 to 36 hours after onset of chest pain and depending on the extent of damage can reach more than 10 times normal levels. In Reye's Syndrome, up to a 70-fold increase in CK activity may be seen due to severe encephalopathy.

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 you Dimension Operator's Guide.

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

Concentration	S.D.
150 U/L	> 7 U/L
800 U/L	> 16 U/L

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

7 – 1000 U/L

14.2 Precision

Material	Mean U/L	Standard Deviation (%CV)	
		Within-run	Total
Serum			
Serum Pool 1	46	0.7	1.3
Serum Pool 2	166	1.4	3.5
Multiqual®			
Level 1	108	1.1	3.5
Level 3	788	3.3	13.4

Form revised 3/31/00

14.3 Interfering Substances

HIL Interference:
The CKI was evaluated for interference according to CLSI/NCCLS EP7-A2. Bias is 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	Test Concentration SI Units	CKI Concentration U/L	Bias %
Hemoglobin (hemolysate)	100 mg/dL	200	<10
Bilirubin	80 mg/dL	200	<10
Lipemia (Intralipid®)	3000 mg/dL	200	<10

14.4 Clinical Sensitivity/Specificity/Predictive Values

Not available.

15. SAFETY

The employee has direct responsibility to avoid injury and illness at work. Nearly all harmful exposures to infectious substances and chemicals, and other injuries, can be avoided with effective training and consistent safe work practices.

Become familiar with the Environmental Health and Safety (EHS) Manual to learn the requirements on working safely and protecting the environment from harm. Although lab work typically focuses on the hazards of working with specimens and chemicals, we must also control other important hazards.

- Slips, trips, and falls cause many serious injuries. Please ensure that spills are cleaned quickly (to avoid slippery floors) and that you can see and avoid obstacles in your path.
- Ergonomic injuries result from performing tasks with too much repetition, force, or awkward position. Ergonomic injuries include strains and back injuries. Learn about ergonomic hazards and how to prevent this type of injury.
- Scratches, lacerations, and needlesticks can result in serious health consequences. Attempt to find ways to eliminate your risk when working with sharp materials.

Report all accidents and injuries immediately to your supervisor or the business unit Environmental Health and Safety Manager or Specialist.

16. RELATED DOCUMENTS

1. Dimension® Clinical Chemistry System Operator's Manual
2. Dimension® Calibration/Verification Procedure
3. Dimension® Cal Accept Guidelines
4. Dimension® Calibration summary
5. Sample Processing, Siemens Dimension® procedure
6. Start up and Maintenance, Siemens Dimension® procedure

7. Laboratory Quality Control Program
8. QC Schedule for Siemens Dimension®
9. Laboratory Safety Manual
10. Material Safety Data Sheets (MSDS)
11. Siemens Dimension® Limits Chart
12. Quest Diagnostics Records Management Procedure
13. Dimension® 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 CKI Flex® Reagent Cartridge DF38

17. REFERENCES

1. Ghoshal, Amit K. and Soldin, Steven J., Evaluation of the Dade Behring Dimension® RxL: Integrated chemistry system-pediatric reference ranges. Clinica Chimica Acta 2003; 331:144
2. Package Insert, CKI Flex® Reagent Cartridge DF38, Siemens Healthcare Diagnostics Inc., 08/28/2008.
3. Package insert, Creatine Kinase Verifier DC26, Siemens Healthcare Diagnostics Inc., 04/2008.
4. Package insert, Liquichek Unassayed Serum Chemistry Controls, Bio-Rad Laboratories, 08/2009.
5. Package insert, Enzyme Diluent, Dimension® Clinical Chemistry System, 03/2008.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes SOP C096.001		
000	5/31/11	10.4	Change CRR upper limit	L. Barrett	J. Buss
000	5/31/11	10.5	Correct all values to match CRR & AMR	L. Barrett	J. Buss
001	6/13/12	10.4	Correct CRR lower limit	J. Buss	J. Buss

19. ADDENDA

None