TRAINING UPDATE

Lab Location:	GEC	**Date Distributed:**	9/7/2018
Department:	Core Lab	**Due Date:**	9/30/2018
		Implementation:	**10/1/2018**

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:

Enzymatic Carbonate (ECO2) by Dimension® Xpand Chemistry Analyzer GEC.C24 v3

Description of change(s):

Added hazard info for Calibrator
Most other changes are format updates

Section	Reason
2	Add more info from PI
5.3	Remove specific calibration steps and reference separate SOP
10.6	Remove repeat value below AMR/CRR
15	Add hazard statement for calibrator
16	Update policy title
17	Update PI dates

This revised SOP will be implemented on October 1, 2018

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

Technical SOP

Title	**Enzymatic Carbonate (ECO_2) by Dimension® Xpand Chemistry Analyzer**
Prepared by	Ashkan Chini Date: 4/12/2011
Owner	Robert SanLuis Date: 4/12/2011

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
Enzymatic Carbonate	Dimension® Xpand Chemistry Analyzer	CO2

Synonyms/Abbreviations
CO_2, ECO_2

Department
Chemistry

2. ANALYTICAL PRINCIPLE

The enzymatic carbonate (ECO2) method for the Dimension® system employs a phosphoenolpyruvate carboxylase-malate dehydrogenase coupled enzymatic reaction and a stable analog of the cofactor NADH. The bicarbonate anion reacts with phosphoenolpyruvate in the presence of phosphoenolpyruvate carboxylase (PEPC) and Mg^{++} to form oxaloacetate and inorganic phosphate (P_i). The oxaloacetate is reduced to malate-by-malate dehydrogenase (MDH) with simultaneous oxidation of the reduced form of an analog (aNADH) of the cofactor, NADH.

$$HCO_3^- + \text{Phosphoenolpyruvate} \xrightarrow{\text{PEPC, } Mg^{++}} \text{Oxaloacetate} + P_i$$

$$\text{Oxaloacetate} + \text{aNADH} + H^+ \xrightarrow{\text{MDH}} \text{Malate} + \text{aNAD}^+$$

The reduction in absorbance of aNADH is proportional to the total CO2 concentration in the sample and is measured biochromatically at wavelengths of 405 nm (primary) and 700 nm (secondary).

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 container or Plastic vial at room temperature

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Criteria		
Stability & Storage Requirements	Room Temperature:	8 hours
	Refrigerated:	(2-8°C) 2 days
	Frozen:	(-20°C or colder) 6 months
Timing Considerations	Total CO2 concentration may be lowered by as much as 6 mmol/L when uncapped specimens are exposed to air for one hour.	
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.	

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
ECO_2	Siemens, Flex® reagent cartridge, Cat. No. DF137

4.2 Reagent Preparation and Storage

Reagent	**Enzymatic Carbonate**
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. • Open well stability: 2 days for wells 1 – 6
Preparation	Reagents are supplied ready for use. No additional preparation is required.

5. CALIBRATORS/STANDARDS

5.1 Calibrators/Standards Used

Calibrator	Supplier and Catalog Number
CHEM III Calibrator	Siemens Dimension®, Cat. No. DC130

5.2 Calibrator Preparation and Storage

Calibrator	CHEM III Calibrator
Preparation	Calibrator is ready for use. No preparation is required.
Storage/Stability	• Store at 2-8ºC • **Unopened**: stable until the expiration date on the label. • **Opened**: once cap is removed, stable for 30 days when recapped immediately after use and stored at 2-8°C

5.3 Calibration Parameter

Criteria	Special Notation
Reference Material	CHEM III Calibrator
Assay Range	5 – 45 mmol/L
Suggested calibration level	See Reagent Package Insert for lot specific assigned values in mmol/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 0.000 C_1 1.000
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

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6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalog Number
Liquichek™ Unassayed Chemistry Controls Levels 1 and 2	Bio-Rad Laboratories Catalog No. 691 and 692

6.2 Control Preparation and Storage

Control	Liquichek™ Unassayed Chemistry Control, Levels 1 and 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	**Thawed**: Open controls are stable for 15 days at 2-8°C. **Frozen**: 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

Step	Action
	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 -20 to -70°C.
- Centrifuge

7.3 Supplies

- Plastic serum tubes and serum cups
- Reagent Grade water (Millipore® or equivalent)
- Calibrated pipettes and disposable tips

8. PROCEDURE

ECO_2 Flex® reagent cartridge Cat. No. DF137 is required to perform this test.

Enzymatic Carbonate is performed on the Dimension Xpand® 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.

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 and processing 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.

8.3	Specimen Testing
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). 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:	5 µL
Reagent 1 Volume:	100 µL
Temperature:	37°C
Wavelength:	405 and 700 nm
Type of Measurement:	Bichromatic rate

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 and prints the concentration of Enzymatic Carbonate in mmol/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

mmol/L

10.4 Clinically Reportable Range (CRR)

5 - 90 mmol/L

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 policy 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…
≤5 mmol/L	Assure there is sufficient sample devoid of bubbles, cellular debris, and/or fibrin clots. Report as: <5 mmol/L
>45 mmol/L	**Manual Dilution**: Using the primary tube, make the smallest dilution possible to bring the raw data within the AMR. Maximum allowable dilution: x 2 **Diluent**: Reagent Grade Water Enter dilution factor as a whole number on the "Enter Sample Data" screen.
>90 mmol/L	If the recommended dilution does not give results within the clinically reportable range, report as: ">90 mmol/L-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

Age	Female / Male
Adult (>18 years):	21 – 32 mmol/L
Pediatric:	
2 – 18 years	21 - 32
13 - 23 months	16 - 25
6 – 12 months	14 - 23
1 – 5 months	13 - 23
7 – 30 days	13 - 22
0 – 6 days	13 - 21

11.2 Critical Values

< 10 mmol/L

11.3 Standard Required Messages

None established

12. CLINICAL SIGNIFICANCE

Increase in serum CO_2 content for the most part reflects increase in serum bicarbonate concentration rather than dissolved CO_2 gas (which accounts for only a small fraction of the total). Increased serum bicarbonate is seen in compensated respiratory acidosis and in metabolic alkalosis. Diuretics (thiazides, ethacrynic acid, furosemide, mercurials), corticosteroids (in long term use), and laxatives (when abused) may cause increased bicarbonate.
Decrease in blood CO_2 is seen in metabolic acidosis and compensated respiratory alkalosis. Substances causing metabolic acidosis include ammonium chloride, acetazolamide, ethylene glycol, methanol, paraldehyde, and phenformin. Salicylate poisoning is characterized by early respiratory alkalosis followed by metabolic acidosis with attendant decreased bicarbonate.

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.
25 mmol/L	> 1.2 mmol/L
50 mmol/L	> 2.0 mmol/L

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

5 – 45 mmol/L

14.2 Precision

Material	Mean mmol/L	Standard Deviation (%CV)	
		Within-run	Total
Dade® Moni-trol® TOTAL Control			
Level 1	13.1	0.5	0.7
Level 2	30.5	0.6	0.9
Plasma Pool	24.5	0.7	1.1
Serum Pool	24.4	0.7	1.1

14.3 Interfering Substances

In rooms with poor ventilation, an open Flex® reagent cartridge well can absorb CO_2 which may cause results to be elevated by up to 30%.
Hemoglobin (hemolysate) of 1000 mg/dL decreases an ECO_2 result of 13 mmol/L by 21%.
Lipemia (Intralipid®) of 3000 mg/dL decreases an ECO_2 result of 13mmol/L by 16%.

HIL Interference:
The ECO_2 method was evaluated for interference from hemolysis, icterus and lipemia according to CLSI/NCCLS EP7-P. 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	Test Concentration SI Units	ECO2 Concentration mmol/L	Bias %
Hemoglobin (hemolysate)	500 mg/dL (monomer)	13	<10
Bilirubin (unconjugated)	80 mg/dL	14	<10
Lipemia (Intralipid®)	1000 mg/dL	13	<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.

ECO2 Flex® Reagent Cartridge is an Irritant. Contains sodium azide as a preservative. Sodium Azide can react with copper or lead pipes in drain lines to form explosive compounds. May cause sensitization by skin contact

CHEM III CAL may cause an allergic skin reaction.
Contains: 5-chloro-2-methyl-3(2h)-isothiazolone mixture with 2-methyl-3(2h)-isothiazolone

Wear protective gloves/protective clothing/eye protection/face protection. 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. Specimen Acceptability Requirements (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 ECO_2 Flex® Reagent Cartridge DF137

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, ECO_2 Flex® Reagent Cartridge DF137, Siemens Healthcare Diagnostics Inc., 03/04/2016.
3. Package insert, CHEM III Calibrator DC130, Siemens Healthcare Diagnostics Inc., 03/2015.
4. Package insert, Liquichek™ Unassayed Chemistry Controls, Bio-Rad Laboratories, 1/2017.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes SOP C062.000		
000	7/15/14	1, 7.1	Add analyzer name	L Barrett	R SanLuis
000	7/15/14	3.2	Add timing for uncapped specimens exposed to air	A Chini	R SanLuis

Version	Date	Section	Reason	Reviser	Approval
000	7/15/14	5, 17	Revised to reflect new CHEM III calibrator	A Chini	R SanLuis
000	7/15/14	6.7	Add use of TEA for lot to lot runs	L Barrett	R SanLuis
000	7/15/14	10.2	Correct rounding to whole number	A Chini	R SanLuis
000	7/15/14	10.5	Remove code QNSR	L Barrett	R SanLuis
000	7/15/14	11.3	Remove priority 3 reporting for SGAH	L Barrett	R SanLuis
000	7/15/14	15	Update to standard wording	L Barrett	R SanLuis
000	7/15/14	16	Update document titles	L Barrett	R SanLuis
000	7/15/14	Footer	Version # leading zero's dropped due to new EDCS in use as of 10/7/13	L Barrett	R SanLuis
1	8/16/16	3.2	Specify anticoagulant	J Negado	R SanLuis
1	8/16/16	4,5,6	Remove individual section labeling instructions and add general one	L Barrett	R SanLuis
1	8/16/16	6.4, 6.5	Replace LIS with Unity Real Time	L Barrett	R SanLuis
1	8/16/16	10.5	Move patient review from section 6	L Barrett	R SanLuis
1	8/16/16	15	Update to new standard wording, add reagent warning from section 4	L Barrett	R SanLuis
1	8/16/16	17	Update PI revision dates	J Negado	R SanLuis
2	8/16/18	2	Add more info from PI	D Collier	R SanLuis
2	8/16/18	5.3	Remove specific calibration steps and reference separate SOP	L Barrett	R SanLuis
2	8/16/18	10.6	Remove repeat value below AMR/CRR	L Barrett	R SanLuis
2	8/16/18	15	Add hazard statement for calibrator	L Barrett	R SanLuis
2	8/16/18	16	Update policy title	L Barrett	R SanLuis
2	8/16/18	17	Update PI dates	D Collier	R SanLuis

19. ADDENDA

None