Brown Clinic Northridge
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BROWN CLINIC
LABORATORY PROCEDURE MANUAL

PROCEDURE: Enzymatic Carbonate

PURPOSE:
The ECO2 Flex® reagent cartridge for the Dimension EXL200 Integrated chemistry system is an *in vitro* diagnostic test intended to quantitatively measure total carbon dioxide in **serum** or **heparinized plasma**.

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 bichromatically at wavelengths of 405 nm (primary) and 700 nm (secondary).

*U.S. patent #5,801,006

INSTRUMENT, REAGENTS & SUPPLIES:
Reagents

Wells[a]	Form	Ingredient	Concentration[b]	Source
1-6	liquid	Potassium Phosphoenolpyruvate	15.6 mM	
		Magnesium Chloride	19.0 mM	
		Sodium Oxamate	1.8 mM	
		3-Acetylpyridine Adenine Dinucleotide	1 mM	
		Phosphoenolpyruvate carboxylase	494 U/L	Bacterial

	Malate Dehydrogenase	5062 U/L	Porcine Heart
	Buffers and stabilizers		

a. Wells are numbered consecutively from the wide end of the cartridge.
b. Represents the nominal value in the final reaction mixture.

Precautions: Used cuvettes contain human body fluids; handle with appropriate care to avoid skin contact and ingestion**.**

Contains sodium azide (<0.1%) as a preservative. Sodium azide can react with copper or lead pipes in drain lines to form explosive compounds. Dispose of properly in accordance with local regulations.

For *in vitro* diagnostic use

Safety Data Sheets (SDS/MSDS) available on www.siemens.com/healthcare

Reagent Preparation: All reagents are liquid and ready-to-use.

REAGENT STORAGE & STABILITY:

Store at 2 – 8° C.

Expiration: Refer to carton for expiration date of individual unopened reagent cartridges. Sealed cartridge wells on the instrument are stable for 30 days. Once wells 1 through 6 have been entered by the instrument, they are stable for two days.

SPECIMEN REQUIREMENTS:

Normal procedures for collecting and storing serum and heparinized plasma may be used for samples to be analyzed by this method.

Samples should be analyzed as promptly as possible after collection and centrifugation of the blood in the unopened tube.

Follow the instructions provided with your specimen collection device for use and processing.

Complete clot formation should take place before centrifugation.

Specimen:

- patient preparation: no patient preparation required
- specimen type: serum or heparinized plasma
- Stability:
  Unopened, separated samples may be stored for 8 hours at room temperature
  Unopened, separated samples may be stored at 2-8^{o}C for 2 days
  Samples may be stored frozen at -20^{o}C or colder for 6 months

  Total carbon dioxide concentration may be lowered by as much as 6 mmol/L when uncapped specimens are exposed to the air for one hour.[4]
  Underfilling of vacutainers may account for low total carbon dioxide results of up to 3 mmol/L.[5]

CONTROLS:

At least once daily run solutions at two levels of a quality control material with known concentrations.

For further details, refer to your Dimension® system manual. The result obtained should fall within limits defined by the day-to-day variability of the system as measured in the user's laboratory. If the results fall outside the laboratory's acceptable limits, follow the procedure in the quality control policy.

PROCEDURE:

Materials Needed

ECO2 Flex® reagent cartridge, DF137

Chem III calibrator, Cat. No. DC130

Test Steps

Sampling, reagent delivery, mixing, processing, and printing of results are automatically performed by the Dimension® clinical chemistry system. For details of this processing, refer to your Dimension® system manual.

Test Conditions

- Sample Size: 5 µL
- Reagent 1 Volume: 100 µL
- Test Temperature: 37° C
- Wavelength: 405 and 700 nm
- Type of Measurement: bichromatic rate

Calibration

The general calibration procedure is described in your Dimension® system manual (also see Appendix B). The following information should be considered when calibrating the ECO2 method:

Assay range: 5 - 45 mmol/L

Reference Material: Chem III calibrator, (Cat. No. DC130).

Suggested Calibration Levels: 0, 25, 50 mmol/L

Calibration Scheme: Three levels in triplicate

Calibration Frequency:
- Every new reagent cartridge lot
- Every 90 days for any one lot
- For each new lot of Flex reagent cartridges
- After major maintenance or service, if indicated by quality control results
- As indicated in laboratory quality control procedures
- When required by government regulations

Assigned Coefficients: C_0 0.000

C_1 1.000

Backup Process:

Refer to Brown Clinic Back-up Policy

INTERPRETATION:
The instrument automatically calculates and prints the concentration of carbon dioxide in mmol/L using the calculation scheme illustrated in your Dimension® system Manual.

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

EXPECTED VALUES:

21 - 32 mmol/L [d.]

Each laboratory should establish its own reference interval for total carbon dioxide as performed on the Dimension® system.

d. This reference interval applies to serum and plasma samples from 120 healthy males and females ages 18 and higher, analyzed using the Dimension® EC02 Flex® reagent method.

REPORTING:

report format: mmol/L

LIMITATIONS:

Results:	>45 mmol/L should be manually diluted.
Manual dilution:	Make appropriate dilution with de-ionized water to obtain result within the assay range. Enter dilution factor.
	Reassay within 15 minutes. Resulting readout is corrected for dilution. Results less than 5 mmol/L should be reported as "less than 5.0 mmol/L" instead of the numerical value.

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® system Manual.

Analytical Specificity

For Known Interfering Substances section refer to package insert.

For Known Non-Interfering Substance refer to package insert.

For Additional Technical Information refer to package insert.

REFERENCES:

ECO2 Flex® reagent cartridge insert sheet PN 71737.101 Issue Date 2013-06-05

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Written By: ____Lori Murray MT(ASCP)_____ Date: _8-8-12

Approved By: _____Aaron Shives, MD_____ Date: ___10/21/2017_____
Laboratory Director

REVIEW - REVISION SUMMARY DOCUMENTATION

Date	By	Revision Summary
07/10	Lori Murray	New format
8-8-12	Lori Murray	Changed instrumentation to EXL200
5/8/15	Heather Hall	Updated information to package insert dated 6/5/2013
10/18/17	Heather Hall	Modified Backup process