POINTE SCIENTIFIC
A MEDTEST COMPANY

Carbon Dioxide (Liquid) Reagent Set

Intended Use
For the quantitative determination of Carbon Dioxide in serum. For *in vitro* diagnostic use only.

Method History
Early methods for the determination of carbon dioxide were based on either volumetric or manometric determination of the CO_2 released from a sample by acid treatment. These methods used the instruments of Van Slyke [1,2] until they were replaced by the Natelson microgasometer,[3] which still uses manometric determination of total CO_2.
Methods have been developed for Auto Analyzers[4] but these suffer from baseline drift[5] and require equipment which many laboratories do not have. Enzymatic methods for CO_2 have been introduced by Wilson,[6] Menson[7] and Norris[8] using phosphoenolpyruvate carboxylase. The present procedure is a enzymatic assay utilizing Phosphoenolpyruvate Carboxylase (PEPC) and a NADH analog.

Principle
$$PEP + HCO_3^- \xrightarrow{PEPC + Mg^{++}} \text{Oxaloacetate} + H_2PO_4^-$$

$$\text{Oxaloacetate} + \text{reduced cofactor} + H^+ \xrightarrow{MDH} \text{Malate} + \text{cofactor}$$

Carbon Dioxide (in the form of bicarbonate ions) reacts with phosphoenolpyruvate (PEP), in the presence of phosphoenolpyruvate carboxylase (PEPC), to form oxaloacetate. The cofactor then in the presence of malate dehydrogenase (MDH) is oxidized by the oxaloacetate. The decrease in absorbance monitored between 405 and 415 nm resulting is proportional to the amount of CO_2 in the sample.

Clinical Significance[5]
The measurement of Carbon Dioxide is useful in the assessment of acid-base balance disturbances. Elevated CO_2 is observed in metabolic alkalosis and compensated respiratory acidosis. Low CO_2 is observed in compensated respiratory alkalosis and metabolic acidosis. Differentiation between the metabolic and respiratory conditions is only possible through additional laboratory determinations.

Reagents
CO_2 reagent: PEP 6mM, Magnesium Ions 10mM, NADH analog, MDH (porcine) ≥ 1200U/L, PEPC (microbial) ≥ 200U/L, Buffer, pH 7.4 ± 0.1 non-reactive stabilizers with surfactants and preservative.

Reagent Preparation
Reagent provided as a ready to use liquid.

Reagent Storage
Reagent is stable until expiration date indicated on vial label when stored tightly capped at 2-8˚C. (15 months from date of manufacture)

Reagent Deterioration
1. Reagent should appear clear and pale yellow in color.
2. Do not use if reagent appears to be turbid, this would indicate deterioration.

Precautions
1. Reagents are for *in vitro* diagnostic use only.
2. Do not ingest. Toxicity has not been established.
3. Do not pipet by mouth to avoid CO_2 contamination from the expired air.

Specimen Collection and Storage
1. Fresh, unhemolyzed serum collected under anaerobic conditions is the recommended specimen.
2. The sample may be stored in ice water under anaerobic conditions for up to one hour.[9]

Interferences
1. Interferences were evaluated for this carbon dioxide method on a Beckman Coulter AU™400 analyzer. No interference was observed by bilirubin up to 20.0 mg/dl and lipemia (intralipid) up to 1000 mg/dl. (Using a criteria of >10% variance from control.) Hemoglobin has been found to interfere with this assay.
2. CO_2 from air or the breath of the analyst is a major interference in this assay. Reagent handling, specimen collection, and all storage instructions must be strictly followed to minimize this interference.
3. A number of conditions and substances have been reported to affect serum Carbon Dioxide levels. [10,11,12]

Materials Provided
Carbon Dioxide Reagent.

Materials Required but not Provided
1. Controls
2. Calibrator
3. Beckman Coulter AU™ analyzer
4. Application and Instrument manuals

Procedure (Beckman Coulter AU™400 application)

SPECIFIC TEST PARAMETERS

TEST NUMBER: **#** TEST NAME: **CO2** ∇ TYPE: **Serum** ∇ OPERATIONAL: **Yes** ∇
SAMPLE VOL.: **2** DIL. VOL.: **0** PRE-DILUTION RATE: **1**
REAGENTS: R1 VOLUME: **200** DIL. VOL.: **0** MIN. OD MAX. OD
R2 VOLUME: DIL. VOL.: **0** L **-2.000** H **2.500**
REAGENT OD LIMIT:
WAVELENGTH: PRI. **410** ∇ SEC. **none** ∇ FIRST L: **-2.000** FIRST H: **2.500**
METHOD: **END** ∇ LAST L: **-2.000** LAST H: **2.500**
REACTION SLOPE: **-** ∇ DYNAMIC RANGE:
MEASURING POINT 1: FIRST: **1** LAST: **27** L: **#** H: **#**
MEASURING POINT 2: FIRST: LAST: CORRELATION FACTOR:
LINEARITY: % A: **1.000** B: **0.000**
NO LAG TIME: ∇ ON BOARD STABILITY PERIOD: **#**

SPECIFIC TEST PARAMETERS

VALUE FLAG: **#** ∇ LEVEL L: **#** LEVEL H: **#**

NORMAL RANGES:	SEX	AGE L YEAR	AGE L MONTH	AGE H YEAR	AGE H MONTH	L	H
○ 1.	# ∇	#	#	#	#	#	#
○ 2.	# ∇	#	#	#	#	#	#
○ 3.	# ∇	#	#	#	#	#	#
○ 4.	# ∇	#	#	#	#	#	#
○ 5.	# ∇	#	#	#	#	#	#
○ 6.	# ∇	#	#	#	#	#	#
7. NONE SELECTED						#	#
8. OUT OF RANGE		L	H			#	#

PANIC VALUE: **#** **#** UNIT: **mmol/L** DECIMAL PLACES: **0**

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CALIBRATION SPECIFIC PARAMETERS

CAL TYPE: **AB** ▽ FORMULA : **Y=AX+B** ▽ COUNTS: **2** PROCESS: **CONC.** ▽

	CAL. NO.	OD	CONC.	FAC/OD-L	FAC/OD-H
POINT 1.	#		#	**-9999999**	**9999999**
POINT 2.					
POINT 3.					
POINT 4.					
POINT 5.					
POINT 6.					
POINT 7.					

1-POINT CAL. POINT: ○ WITH CONC-0

MB TYPE FACTOR: CALIBRATION STABILITY PERIOD: **#**

#: User-Defined

The above reagent parameters are intended to serve as a guide for use with Pointe Scientific, Inc. reagent. The parameters are based on data generated by Pointe Scientific, Inc. Please note: These parameters should be used in conjunction with your laboratory Quality Control Program for validation.

NOTE: For other instrument specific applications please contact Pointe Scientific, Inc. Technical Service Department at 1-800-445-9853

Limitations

1. Samples exceeding 40 mmol/L must be diluted 1:1 with saline, re-assayed, and the result multiplied by two.
2. Carbon Dioxide contamination must be avoided. Keep reagent tightly capped when not in use.

Calibration

The procedure should be calibrated according to the instrument manufacturer's calibration instructions. If control results are found to be out of range, the procedure should be recalibrated.

Calculation

$$\frac{\text{Abs. Sample}}{\text{Abs. Standard}} \times C_{st} = \text{Carbon Dioxide}$$

Where C_{st} = Value of Standard in mmol/L

Sample Calculation:
If Abs. Standard = 0.250, Abs. Sample = 0.225 and concentration of Standard = 30 mmol/L then:

$$\frac{0.225}{0.250} \times 30 \text{ mmol/L} = 27 \text{ mmol/L}$$

Quality Control

To monitor the reliability of results, two levels of control sera with known Carbon Dioxide values should be run with patient samples.

Expected Values [9]

23-34 mmol/L
It is strongly recommended that each laboratory determine its own reference range.

Performance

1. Assay Range: 2 - 40 mmol/L
2. Comparison: A comparison study performed between the Beckman Coulter AU400 and Hitachi 717 using this method resulted in a correlation coefficient of $y = 1.068x - 0.4$. $r = 0.978$ (N=35)
3. Precision: Within Day precision was investigated by running two samples in replicates of 20 on the same day. Between - day precision study was performed using two levels of control material with 2 runs per day and 2 replicates per run.

Within Day (n=20)			Day to Day (n=20)		
Mean	**S.D.**	**C.V.%**	**Mean**	**S.D.**	**C.V.%**
10.7	0.6	5.6	8.6	0.4	4.7
30.3	0.8	2.6	27.2	1.3	4.8
42.0	0.7	1.7			

Precision and Linearity studies were performed following modifications of CLSI Protocols EP5 and EP6[13] using a Beckman Coulter AU™400 analyzer

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EC REP

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Rev. 03/15 P803-OC702-01