MEDTEST DX
A MEDTEST COMPANY

Carbon Dioxide (Liquid) Reagent Set

Intended Use
For the quantitative determination of Carbon Dioxide in serum using the Mindray BS-480 analyzer. 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
$$PEP + HCO_3^- \xrightarrow{PEPC + Mg^{++}} Oxaloacetate + H_2PO_4^-$$

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

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 and Stability
Reagent is stable until expiration date indicated on vial label when stored tightly capped at 2-8˚C. (15 months from date of manufacture). Manufacturer studies have shown reagent is stable for 30 days once placed in the refrigerated reagent carousel (2-10°C), however reagent stability may vary based on individual laboratory conditions.

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 and Hazards
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.

Hazards:
Hazard Classifications: Not a hazardous substance or mixture.
Pictogram: Not required.
Signal Word: Not required.
Hazard Statements: Not a hazardous substance or mixture.
Precautionary Statements: Not a hazardous substance or mixture
Refer to the Safety Data Sheet for this product (SDS-CO2600) available at www.medtestdx.com.

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 Mindray BS-480 analyzer. No interference was observed by bilirubin up to 20.0 mg/dl, hemoglobin up to 219.9 mg/dl and lipemia (intralipid) up to 1000 mg/dl. (Using a criteria of >10% variance from control. CO_2 level was 26, 23, 25 mmol/L respectively)
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]

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Materials Provided
Carbon Dioxide Reagent

Materials Required but not Provided
1. Mindray BS-480 Analyzer
2. BS-480 Operation manual
3. Chemistry Calibrator, catalog number CHEC480
4. Chemistry control, catalog number CHEQ480

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
Use an NIST-traceable serum calibrator. The procedure should be calibrated according to the instrument manufacturer's calibration instructions. If control results are found to be out of range, the test may need to be re-calibrated. Under typical operating conditions manufacturer calibration stability studies have shown the calibration curve will be stable for at least 1 day.

Quality Control
To monitor the reliability of results, two levels of control sera with known Carbon Dioxide values should be run with patient samples. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.

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 study was performed between the Mindray BS-480 and a similar analyzer using this method, resulting in the following:

Method	Carbon Dioxide
N	97
Mean CO2 (mmol/L)	22.5
Range (mmol/L)	4-39
Standard Deviation	6.4
Regression Analysis	y = 0.962x – 2.2
Correlation Coefficient	0.9543

3. Precision: Within Day precision was investigated by running two samples in replicates of 20 on the same day. Day to Day results were obtained by performing one run per day over a span of 20 days. Precision studies were performed using the Mindray BS-480 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.[13]

Within Day

Sample	LOW	MID	HIGH
N	20	20	20
Mean	7.8	23.9	30.9
Standard Deviation	0.4	0.5	0.6
Coefficient of Variation (%)	5.7%	2.1%	2.0%

Total

Sample	LOW	MID	HIGH
N	40	40	40
Mean	10.0	22.6	29.4
Standard Deviation	1.1	2.0	1.0
Coefficient of Variation (%)	10.5%	8.7%	3.3%

4. Sensitivity: 2SD limit of detection (95% Conf) = 1 mmol/L

References

1. Van Slyke, D.D. and Stadie, W.C., J. Biol. Chem. 49:1 (1921).
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3. Natelson, S., Microtechniques of Clinical Chemistry, C. Thomas, Springfield, IL. P.147 (1961).
4. Skeggs, L.T. Jr., Am. J. Clin. Path. 33:181 (1960).
5. Tietz, N.W., Fundamentals of Clinical Chemistry, W.B. Saunders, Philadelphia, PA., pp 884-887 (1982).
6. Wilson, W., et al, Clin. Chem. 19:640 (1973).
7. Menson, R.C., et al, Clin. Chem. 20:872 (1974).
8. Norris, K.A., et al, Clin. Chem. 21:1093 (1975).
9. Henry, R.J., Clinical Chemistry: Principles and Technics, Harper & Row, New York, NY, p784 (1974).
10. Young, D.S., et al, Clin. Chem. 21:1D (1975).
11. Martin, E.W., In Hazard of Medication (Alexander, S.F., Farage, D.J., and Hassan, W.E., Jr. eds.), J.B. Lippincott Co., Philadelphia, PA., and Toronto, Canada, p. 169 (1971).
12. Constantino, N.V., and Kabat, H.F., Am. J. Hosp. Pharm. 30:24 (1973).
13. NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Ed. (1992)

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CHEMISTRY PARAMETERS

Chem:	CO2	No.:	208	Sample Type:	Serum
Chemistry:	Carbon Dioxide			Print Name:	CO2
Reaction Type:	Fixed Time			Reaction Direction:	Negative
Pri Wave:	412			Sec Wave:	505
Unit:	mmol/L			Decimal	0
Blank Time:	10 12			Reaction Time:	18 41

	Sample Vol.	Aspirated	Diluent	Reagent Vol.	Diluent
Standard:	1.5 ul	--- ul	--- ul	R1: 150 ul	--- ul
Decreased:	--- ul	--- ul	--- ul	R2: --- ul	-- ul
Increased:	--- ul	--- ul	--- ul	R3: --- ul	-- ul
	☐ Sample Blank	☑ Auto Rerun		R4: --- ul	--- ul

Slope/Offset Adjustment

Slope: 1 Offset: 0

Linearity Range (Standard)	2	40		Linearity Limit:
Linearity Range (Decreased)	____	____		Substrate Depletion:
Linearity Range (Increased)	____	____		Mixed Blank Abs:
R1 Blank Abs:	____	____		Uncapping Time
Blank Response:	____	____		Reagent Alarm Limit:
Twin Chemistry:				☐ Enzyme Linear Extension

☐ Prozone Check ○ Rate Check ○ Antigen Addition

Q1: Q2: Q3: Q4:

PC: ABS:

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CALIBRATION PARAMETERS

Calibrator Definition

Calibrator:	*	Lot No.:	*
Exp Date:	*		

Carousel	**Pos**
Sample Carousel 1	*
Sample Carousel 2	
Sample Carousel 3	

Reagent/Calibration

Calibrator	Pos	Lot No	Exp Date	Chem	Conc	Unit
Water	W	*	*	CO2	0	mmol/L
Chemistry Calibrator	*	*	*	CO2	*	mmol/L

Calibration Setup

Chem: CO2

Calibration Settings

Math Model:	Two-Point Linear		
Factor:		Replicates:	2

Acceptance Limits

Cal Time:	*	Hour	
Slope Diff:	---	SD:	---
Sensitivity :	---	Repeatability:	---
Deter Coeff:	---		

Auto Calib.

☐ Bottle Changed ☐ Lot Changed ☐ Cal Time

It is recommended that two levels of control material be assayed daily.
* Indicates user defined parameter.

REF CO2480

Manufactured for MedTest DX
5449 Research Drive Canton, MI 48188

2°C 8°C

IVD

Symbol Key

Use by (YYYY-MM-DD)	LOT Lot and batch code	REF Catalog number	Manufacturer
Temperature limitation	Consult instructions for use	IVD *In vitro* diagnostic medical device	