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Carbon Dioxide (Liquid) Reagent Set

Intended Use
For the quantitative determination of Carbon Dioxide in serum using the Mindray BS-200 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
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 Hitachi 917 analyzer. No interference was observed by bilirubin up to 20.0 mg/dl, hemoglobin up to 400 mg/dl and lipemia (intralipid) up to 1000 mg/dl. (Using a criteria of >10% variance from control. CO_2 level was 19mmol/L)
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. Mindray BS-200 Analyzer
2. BS-200 Operation manual
3. Chemistry Calibrator, catalog number C7506-50
4. Chemistry control, catalog number C7592-100

Mindray BS-200 Test Parameters

Test :	CO2	R1 :	300
No.:	011	R2 :	0
Full Name:	Carbon Dioxide	Sample Volume:	3
Standard No.:		R1 Blank:	
Reac. Type:	Fixed-time	Mixed Rgt. Blank:	
Pri. Wave:	405nm	Linearity Range:	2 - 40
Sec. Wave:	510nm	Linearity Limit:	
Direction:	Decrease	Substrate Limit:	
Reac. Time:	2 / 15	Factor:	
		Compensate: Slope 1.0	Intercept: 0
Incuba. Time:	0	□ Prozone check	
Unit:	mmol/L	q1: q2: q3: q4:	
Precision:	Integer	PC: Abs:	

Calibration Parameters

Rule:	Two-point linear	Calibrator 1:	Deionized Water
Sensitivity:		Calibrator 2:	Chem Cal
Replicates:	2	Calibrator 3:	
Interval (day):		Calibrator 4:	
Difference Limit:		Calibrator 5:	
SD:		Calibrator 6:	
Blank Response:			
Error Limit:			
Coefficient:	0		

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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 procedure should be re-calibrated.

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

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. 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. Low Limit of Detection: 2 mmol/L
3. Comparison: A study was performed between the Mindray BS-200 and a similar analyzer using this method, resulting in a correlation coefficient of 0.982 and a linear regression equation of y = 0.934x + 1.7. (N=35,)
4. 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-200 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.[13]

Within Day (n=20)			Day to Day (n=20)		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
14.1	0.2	1.6	14.6	0.6	4.1
22.4	0.5	2.2	21.4	0.9	4.2

References

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Rev. 12/13 M803-CO2600-01

Use by (YYYY-MM) | LOT Lot and batch code | REF Catalog number | Manufacturer | IVD *In vitro* diagnostic medical device
Temperature limitation | Consult instructions for use | CE mark | EC REP Authorized representative in the European Community