

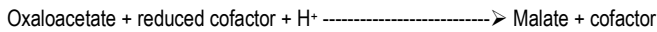
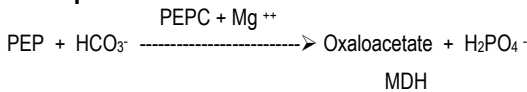
Intended Use

For the quantitative determination of Carbon Dioxide in serum using the Yumizen C230 and Yumizen C240 analyzers. For *in vitro* diagnostic use only. **Rx Only.**

Method History

Early methods for the determination of carbon dioxide were based on either volumetric or manometric determination of the CO₂ 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 CO₂. Methods have been developed for Auto Analyzers⁴ but these suffer from baseline drift⁵ and require equipment which many laboratories do not have. Enzymatic methods for CO₂ have been introduced by Wilson,⁶ Menson⁷ and Norris⁸ using phosphoenolpyruvate carboxylase. The present procedure is an enzymatic assay utilizing Phosphoenolpyruvate Carboxylase (PEPC) and a NADH analog.

Principle



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 CO₂ in the sample.

Clinical Significance⁵

The measurement of Carbon Dioxide is useful in the assessment of acid-base balance disturbances. Elevated CO₂ is observed in metabolic alkalosis and compensated respiratory acidosis. Low CO₂ is observed in compensated respiratory alkalosis and metabolic acidosis. Differentiation between the metabolic and respiratory conditions is only possible through additional laboratory determinations.

Reagents

CO₂ 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 CO₂ 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. CO₂ level was 19mmol/L)

2. CO₂ 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. Yumizen C230 / Yumizen C240 Analyzer
2. Yumizen C230 / Yumizen C240 Operation manual
3. Chemistry Calibrator, catalog number C7506-50
4. Chemistry control, catalog number C7592-100

Test Parameters

Test:	CO2	Chemistry:	Carbon Dioxide
Chemistry No.:	208	Print Name:	CO2
Reaction Type:	Fixed-Time	Reaction Direction:	Negative
Pri. Wave:	405 nm	Sec. Wave:	510nm
Decimal.:	0	Samp. Type:	Serum
Blank Time:		Reaction Time:	2 15
Unit:	mEq/L	Incubation Time:	0

	Sample Vol.	Aspirated	Diluent	Reagent Vol.	Diluent
Standard;	2	uL	uL	R1: 200	uL uL
Decreased;		uL	uL		
Increased;		uL	uL		

Linearity Range (Standard);	2-40	Linearity Limit:	
Linearity Range (Decreased):		Substrate Depletion:	
Linearity Range (Increased):		Mixed Blank Abs.:	- 40000 40000
R1 Blank Abs.:	- 40000 40000	On-board Stability:	Day (s)
Blank Response	- 40000 40000	Reagent Alarm Limit:	3
Twin Chemistry:			

Prozone Check:		
Q1:	Q2:	Q3:
Q4:	PC:	ABS:

Use Qualitative Result:	Range:	Flag:
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Slope Offset:	Slope	Offset	Unit
	1	0	mEq/L

Pretreatment:		
Pretreat Sample Vol.:	uL	Pretreat Reagent Vol.:
		uL

Ref. Range:			
Sample Type:	Gender:	Age Range:	Ref. Range: Critical Range: Unit:

Calibration Setup Parameters

Chem:	CO2	Calibrator	Conc.	Pos	Lot No.
Calibration Setting		Water	0.0	W	
Math Model:	Two-Point Linear	Chem Cal	*	*	
Factor:	Replicates: 2				
Acceptance Limits					
Cal Time:	24 hr.				
Slope Diff:	SD:				
Sensitivity:	Repeatability:				* User Defined
Deter Coeff:					
Auto Calib.					
	<input type="checkbox"/> Cal Time				

Pointe Carbon Dioxide Reagent Set

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)

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

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 Yumizen 200 series 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 Yumizen 200 series 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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4. Skeggs, L.T. Jr., Am. J. Clin. Path. 33:181 (1960).
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6. Wilson, W., et al, Clin. Chem. 19:640 (1973).
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13. NCCLS document "Evaluation of Precision Performance of Clinical Chemistry Devices", 2nd Ed. (1992)

Symbol Key

Use by (YYYY-MM-DD)	Lot and batch code
Catalog number	Manufacturer
In vitro diagnostic medical device	Temperature limitation
Consult instructions for use	Rx Only: Prescription Use Only
CE mark	Authorized representative in the European Community

12-C7502-160	Manufactured by HORIBA Instruments Incorporated - Pointe Brand 5449 Research Drive Canton, MI 48188		
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Manufactured by HORIBA Instruments Incorporated – Pointe Brand
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Certified to Perform Reagents

The Pointe reagents are certified to be manufactured according to specified parameters. Any Pointe reagent product not meeting specifications through its listed expiration date will be remedied immediately without charge.