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Carbon Dioxide Enzymatic Assay (405nm)

Catalog Number: BQ007-EAEL Size: 5 x 20mL
BQ007-EACL Size: 1 x 2mL

INTENDED USE

For the in vitro quantitative determination of Carbon Dioxide content in human serum or plasma.

CLINICAL SIGNIFICANCE

Serum CO₂ is a blood test that measures the amount of carbon dioxide (CO₂) in serum. Serum CO₂ is really a measure of serum HCO₃⁻, also called bicarbonate. In the body, 95% of the CO₂ is present as HCO₃⁻, so most of what is measured in the laboratory represents HCO₃⁻.

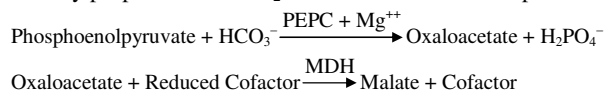
Higher-than-normal levels of HCO₃⁻ may indicate excessive vomiting, respiratory dysfunction (breathing disorders), hyperaldosteronism, or Cushing syndrome.

Historic procedures to measure HCO₃⁻ in the laboratory usually involve addition of acid to liberate CO₂, followed by measurement by volumetric, manometric, thermal conductivity or GC/MS, or ISE methods. These procedures are both time consuming and cumbersome. Bio-Quant Carbon Dioxide Enzymatic Assay is a quick, easy to use enzymatic procedure applicable to routine laboratory instrumentation.

The CO₂ levels in the blood are influenced by kidney and respiratory (lung) function. Lower-than-normal levels of HCO₃⁻ may indicate ketoacidosis, lactic acidosis, kidney disease, diarrhea, methanol poisoning, salicylate toxicity (such as aspirin overdose), ethylene glycol poisoning, or Addison disease (adrenal gland insufficiency).

ASSAY PRINCIPLE

Bio-Quant Carbon Dioxide Enzymatic Assay is based on two coupled enzyme reactions including phosphoenolpyruvate carboxylase (PEPC) and malate dehydrogenase (MDH). PEPC catalyzes the first reaction which produces oxaloacetate. In the presence of MDH, the reduced cofactor is oxidized by oxaloacetate. This results in a decrease of absorbance at 405 or 415 nm that is directly proportional to CO₂ concentration in the sample.



REAGENT COMPOSITION

Reagent R1: PEP, PEPC, NADH and MDH in buffer
Calibrator: 30mM Sodium Bicarbonate in 0.9% Saline

REAGENT PREPARATION

Bio-Quant Carbon Dioxide Enzymatic Assay Reagent (R1) is a ready to use, single liquid reagent.

REAGENT STABILITY AND STORAGE

Unopened reagents are stable until the expiration date printed on the outer box.

MATERIALS REQUIRED BUT NOT PROVIDED

Any instrument with temperature control of 37 ± 0.5°C that is capable of reading absorbance accurately at 405 or 415 nm may be used. Application sheets for use of Bio-Quant Carbon Dioxide Enzymatic Assay on automated clinical chemistry analyzers are attached to the end of this insert. If you do not see your machine listed please call 858-450-0048 or email to: info@bqkits.com.

SPECIMEN COLLECTION AND PREPARATION

Serum or heparinized plasma may be assayed. Ideally, venous blood should be collected and handled anaerobically. Do not use citrate or oxalate as anticoagulant.

Plasma and serum, after prompt separation from cells or clot, should be kept tightly stoppered. CO₂ content of blood is stable for 1 hour when stored at 2–4°C under anaerobic conditions.

PRECAUTIONS

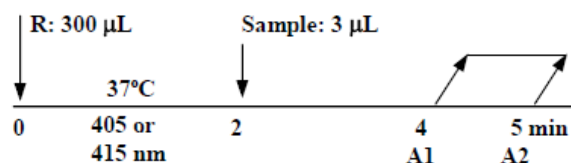
1. For in vitro diagnostic use only.
2. Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 93-8395).
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. Avoid ingestion and contact with skin and eyes. See Material Safety Data Sheet.
5. The reagent contains <0.1% sodium azide, NaN₃, as preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azide. On disposal, flush with a large volume of water to prevent azide buildup.
6. Avoid contamination of the reagent with CO₂. Do not blow into pipette, since breath contains a high content of CO₂. Do not let bottles remain open unnecessarily, since CO₂ from air can contaminate the reagent. Keep container tightly stoppered.
7. Do not use the reagents after the expiration date labeled on the outer box.
8. The reagent solution should be clear. If turbid, the reagent may have deteriorated.

ASSAY PROCEDURE

Conditions

Please see attached data sheet for instrument-specific parameters.

TEST SCHEME FOR CHEMISTRY ANALYZERS



CALIBRATION

A carbon dioxide calibrator is included with the reagent and, along with 0.9% saline as a zero reference, should be used as directed to calibrate the procedure.

QUALITY CONTROL

We recommend that each laboratory use carbon dioxide controls to validate the performance of carbon dioxide reagents. A set of normal and abnormal range carbon dioxide controls is available from Bio-Quant Laboratories (Cat. # BQ007-EACN). If the results from the controls fall outside the acceptable limits, as determined by the manufacturer, the test should not be performed. We recommend that your quality control testing follows federal, state, and local guidelines or accreditation requirements.

Results

Carbon dioxide concentration is expressed as mmol/L (mEq/L).

LIMITATIONS

A sample with a carbon dioxide level exceeding the linearity limit should be diluted with 0.9% saline and reassayed incorporating the dilution factor in the calculation of the value.

REFERENCE RANGE

Normal values of CO₂ in serum or plasma are 22–29 mmol/L for adults and 20-28 mmol/L for infants and children.⁴ It is strongly recommended that each laboratory establish an expected range characteristic for the local population.

PERFORMANCE CHARACTERISTICS

These performance characteristics were determined at Bio-Quant Laboratories using automated procedures on Olympus AU400.

LIMIT OF DETECTION

The limit of detection is 1.2 mmol/L. Sensitivity was calculated on 12 replicates of normal saline and reported as the “mean zero value + 3 SD.”

INTERFERENCE

Interference for the Bio-Quant Carbon Dioxide Enzymatic Assay was evaluated on a Cobas Mira analyzer. The following substances normally present in serum produced less than 10% deviation at the listed concentrations: Triglycerides at 1000 mg/mL, Ascorbic acid at 5 mg/mL, Bilirubin at 40 mg/mL, Bilirubin Conjugated at 40 mg/mL, and Hemoglobin at 200 mg/mL

LINEARITY

The linearity of the procedure is from 1.12 to 50 mmol/L.

PRECISION

The precision of the Bio-Quant Carbon Dioxide Enzymatic Assay was evaluated on the Cobas Mira instrument according to Clinical Laboratory Standards Institute (formerly NCCLS) EP5-A guideline. In the study, two specimens containing 25mM and 40mM CO₂ were tested with 2 runs per day with duplicates over 20 working days.

No. of Data Points	Within Run Precision		Run to Run Precision	
	25mM CO ₂	40mM CO ₂	25mM CO ₂	40mM CO ₂
80	80	80	80	80
Mean (mM)	24.1	40.1	24.1	40.1
SD (mM)	0.56	0.91	0.68	1.32
C _v %	2.3%	2.3%	2.8%	3.3%

Additionally, the precision of the Bio-Quant Carbon Dioxide Enzymatic Assay was evaluated on the Cobas Mira instrument using samples in the abnormal low range. In the study, 20 specimens ranging from 15.39 – 16.96mM CO₂ were tested in 3 runs on 2 working days, resulting in a Mean of 16.1mM, an SD of 0.275mM and a C_v% of 1.70%.

ACCURACY

The performance of this assay was compared with the performance of a similar carbon dioxide assay on a Cobas Mira analyzer using serum and plasma samples.

Sixty serum samples ranging from 5.9 – 44.5 mmol/L gave a correlation coefficient of 0.9859. Linear regression analysis gave the following equation:

This method = 1.0447 (reference method) – 0.9742 mmol/L

Sixty plasma samples ranging from 3.73 - 40.46 mmol/L gave a correlation coefficient of 0.9731. Linear regression analysis gave the following equation:

This method = 0.9863 (reference method) + 0.1486 mmol/L

REFERENCES

1. Tietz, N. W. (Ed): Fundamentals of Clinical Chemistry, W. B. Saunders Co., Philadelphia, 865 (1982)
2. Contarow and Trumper, Clinical Biochemistry, 7th ed., Al Latner, Editor, Saunders, Philadelphia, p. 399 (1975)
3. Clinical Chemistry, LA Kaplan, AJ Pesce, Editors, CV Mosby Company, St. Louis (MO), p. 1056 (1984)
1. Burtis CA, Ashwood ER, eds. Tietz Textbook of Clinical Chemistry, 2nd ed. Philadelphia, PA: WB Saunders, p. 2181 (1994)

Instrument Settings for Olympus AU400 at 37°C

General	LIH	ISE	Range	
Test Name:	9 CO ₂	Type:	Serum	Operation: Yes
Sample:	Volume 3.0 μL	Dilution	0 μL	Pre-Dilution Rate: 1
Reagents:	R1 volume 300 μL	Dilution	0 μL	Min OD Max OD
	R2 volume 0 μL	Dilution	0 μL	L: <input type="checkbox"/>
Wavelength:	Pri. 410	Sec. 520	Reagent OD Limit:	
Method:	END1	First L:	-2.000	First H:
	2.500	Last L:	-2.000	Last H:
Reaction Slope:	<input type="checkbox"/>	Dynamic Range:		
Measuring Point 1:	First 2	Last 18	L: <input type="checkbox"/> 0.00 H:	
Measuring Point 2:	First <input type="checkbox"/>	Last <input type="checkbox"/>	1000.00	
Linearity	<input type="checkbox"/>	%	Correlation Factor:	
No-Lag-Time:	<input type="checkbox"/>	A:	1.000000	B:
	0.000000	Onboard stability Period		
	999			

Instrument Settings for Hitachi 717 at 37°C

CHEMISTRY PARAMETERS

TEST	[CO ₂]
ASSAY CODE	[2POINT]:[3]-[24]
SAMPLE VOLUME	[3][3]
R1 VOLUME	[300][100][NO]
R2 VOLUME	[0][100][NO]
WAVELENGTH	[505][405]
CALIB. METHOD	[LINEAR] [0][0]
STD. (1) CONC.-POS	[0.0]-[1]
STD. (2) CONC.-POS	[30.0]-[2]
STD. (3) CONC.-POS	[*.*]-[*]
STD. (4) CONC.-POS	[*.*]-[*]
STD. (5) CONC.-POS	[*.*]-[*]
STD. (6) CONC.-POS	[*.*]-[*]
SD LIMIT	[50]
DUPLICATE LIMIT	[32000]
SENSITIVITY LIMIT	[0]
ABS. LIMIT (INC/DEC)	[0][DECREASE]
PROZONE LIMIT	[0][LOWER]
EXPECTED VALUE	[0]-[100]
TECH. LIMIT	[-100]-[1000]
INSTRUMENT FACTOR	[1.0]
***	1:1POINT 2:2POINT 3:3POINT
	4:1POINT&R 5:RATE-A 6:RATE-B

* DATA ENTERED BY OPERATOR

Instrument Settings for COBAS MIRA at 37°C

P2 TESTS ROUTINE
CO2 SYSE/17-FEB-06

GENERAL

MEASUREMENT MODE : ABSORB
REACTION MODE : R-S
CALIBRATION MODE : SLOPE AVG
REAGENT BLANK : REAG/SOL
CLEANER : NO

WAVELENGTH : 405 nm
DECIMAL POSITION : 2
UNIT : mmol/l

ANALYSIS

POST DIL. FACTOR : NO
CONC. FACTOR : NO

SAMPLE CYCLE : 1
VOLUME : 3.0 ul
DILUTION NAME : H2O
VOLUME : 0.0 ul

REAGENT CYCLE : 1
VOLUME : 300 ul

CALCULATION

SAMPLE LIMIT : NO

REAC. DIRECTION : DECREASE
CHECK : OFF

CONVERS. FACTOR : 1.0000
OFFSET : 0.0000

TEST RANGE LOW : NO
RANGE HIGH : NO
NORM. RANGE LOW : NO
RANGE HIGH : NO

NUMBER OF STEPS : 1

CALC. STEP A : END-POINT
READINGS FIRST : 2 LAST: 14

CALIBRATION

CALIB. INTERVAL : EACH RUN

BLANK SOL-POS : 8
REAG. RANGE LOW : NO
HIGH : NO
BLANK RANGE LOW : NO
HIGH : NO

STANDARD POS : 1
STD-1 : 30.0 MMOL/L
STD-2 : NO
STD-3 : NO

REPLICATE : DUPL
DEVIATION : NO

CONTROL

CS1 POS: NO
CS2 POS: NO
CS3 POS: NO