

Carbon Dioxide (340nm)

1.0 INTENDED USE

This reagent is intended for the quantitative determination of carbon dioxide (CO₂) in serum.

2.0 BACKGROUND

2.1 METHOD AND 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 (10.1, 10.2) until they were replaced by the Natelson microgasometer, (10.3) which still uses manometric determination of total CO₂.

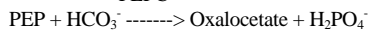
Methods have been developed for Auto Analyzers (10.4) but these suffer from baseline drift (10.5) and require equipment which many laboratories do not have.

Enzymatic methods for CO₂ have been introduced by Wilson, (10.6) Menson (10.7) and Norris (10.8) using phosphoenolpyruvate carboxylase.

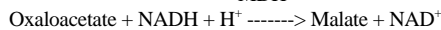
This procedure is based on their work.

2.2 TEST PRINCIPLE

PEPC



MDH



Carbon Dioxide (in the form of bicarbonate ions) reacts with phosphoenol-pyruvate (PEP), in the presence of phosphoenol-pyruvate carboxylase (PEPC) to form oxaloacetate and phosphate. The oxaloacetate is then converted to malate by the action of malate dehydrogenase (MDH) and reduced nicotinamide adenine dinucleotide (NADH). The decrease in absorbance at 340nm resulting from the oxidation of NADH is proportional to the amount of CO₂ in the sample. Interference from endogenous pyruvate and LDH is eliminated by the inclusion of sodium oxamate.

2.3 CLINICAL SIGNIFICANCE (10.5)

The measurement of CO₂ 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.

3.0 SPECIMEN COLLECTION AND HANDLING

3.1 PATIENT PREPARATION

No special patient preparation is required.

3.2 SPECIMEN COLLECTION

Fresh, unhemolyzed serum collected under anaerobic conditions is the recommended specimen. Heparinized plasma collected under anaerobic conditions is acceptable. Oxalate, citrate, and EDTA should not be used as they cause shifts of electrolytes and water between plasma and cells. Samples which are lipemic, icteric, or hemolyzed may be used if a sample blank is performed. (See 5.5 PROCEDURE NOTES.)

Use a standard venipuncture tube to draw patient sample.

The amount of sample required will depend on the analyzer used. Call Biotron's technical service department at 1-800-5958766 for the recommended sample volume for your analyzer.

Record the patient's name, date and time of sample collection and preparation.

3.3 SPECIMEN STORAGE

The sample may be stored in ice water under anaerobic conditions for up to one hour (10.9.) It is recommended that testing be done as soon as possible after sample collection and preparation. Otherwise store the sample properly using the guidelines above.

4.0 MATERIALS

(10 X 10 ml)

Reagents necessary for the determination of carbon dioxide are included in the kit.

4.1 REAGENT

CO₂ reagent contains after reconstitution:

PEP	1.8 mM
Magnesium Sulfate	10 mM
NADH	0.40 mM
MDH (porcine)	≥ 1200 U/L
PEPC (microbial)	≥ 200 U/L
Sodium oxamate	2.5 mM
Buffer (pH 8.0 ± 0.1)	50 mM
Sodium Azide as preservative	0.1%

4.2 WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use. Not for Internal use in Humans or Animals. In Vitro Diagnostics reagents may be hazardous. Avoid ingestion and skin or eye contact.

The reagent contains sodium azide at 0.1%. This may react with copper or lead plumbing to form explosive metal azides. Upon disposal, flush with large volume of water to prevent azide build up.

4.3 REAGENT PREPARATION

Reconstitute the reagent with 10ml of CO₂ diluent. Replace the rubber stopper and allow 5 minutes for reconstitution. Swirl gently until the contents of the vial are completely dissolved. Record the date and time of reconstitution.

Note: CO₂ diluent should be used as provided. Do not shake or mix the diluent vial.

4.4 REAGENT STORAGE AND STABILITY

Unreconstituted reagent should be stored at 2-8°C and is stable until the expiration date on the label. Do not use the reagent if there is evidence that moisture has entered the vial, such as caking or incomplete dissolution.

Reconstituted reagent is stable for 24 hours at room temperature and 7 days at 2-8° C. Keep tightly capped at all times and avoid excessive shaking. Do not use the reagent if the absorbance of the reagent is less than 0.700 at 340 nm.

4.5 ADDITIONAL MATERIALS REQUIRED

- 4.5.1 Spectrophotometer or colorimeter capable of reading absorbance at 340 nm.
- 4.5.2 1 cm cuvettes or flow cell capable of transmitting light at 340 nm.
- 4.5.3 Test tubes capable of holding 2 ml; cylinders for preparing the working reagent.
- 4.5.4 Pipettes capable of delivering 1 ml and 10 µl.
- 4.5.5 Distilled or deionized water or CO₂ diluent for reconstituting the reagent.
- 4.5.6 Constant temperature source which can be adjusted to 37° C.
- 4.5.7 CO₂ standard or calibrator.
- 4.5.8 Normal and abnormal controls for quality control.

5.0 TEST PROCEDURE

The following is a general procedure for use on a manual instrument.

5.1 PROCEDURE CONDITIONS

Wavelength	340 nm
Temperature	37° C
Pathlength	1.0 cm
Mode	kinetic
Reaction time	5 min
Sample volume	10 µl
Reagent volume	1.0 ml
Total volume	1.010 ml
Sample to reagent ratio	1/100

5.2 INSTRUMENT

Any instrument capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 340 nm may be used. The band width should be 10 nm or less, stray light 0.5% or less, and the wavelength accuracy within 2 nm.

5.3 CALIBRATION

Use an aqueous CO₂ standard or a serum based calibrator. The assay is calibrated by referencing the absorbance of the unknown sample to the absorbance of the standard (or calibrator).

5.4 PROCEDURE

5.4.1 Prepare the required volume of working reagent. (See 4.3 Reagent Preparation Section.)

5.4.2 Label tubes as "blank", "standard" and "patient".

5.4.3 Add 1.0 ml of working reagent in each tube.

5.4.3 Pipette 10 µl of standard (or calibrator) and patient into their respective tubes. Mix, and incubate for 5 minutes 37° C.

5.4.4 Zero the photometer at 340 nm using distilled water.

5.4.5 Incubate for 45 seconds and record the absorbance at 340 nm of the patient (A1), standard (As1) and the reagent blank (Ab1).

5.4.6 After exactly 30 seconds record the absorbance at 340 nm of the patient (A2), standard (As2) and the reagent blank (Ab2).

5.5 PROCEDURE NOTES

CO₂ from air or the breath of the analyst is a major interference in this assay. Reagent preparation, specimen collection, and all storage instructions must be strictly followed to minimize this interference.

If the sample is very lipemic, icteric or hemolyzed, a sample blank must be prepared by adding 10 µl of sample to 1 ml of saline. The absorbance of this blank is subtracted from the absorbance of the respective test and the corrected absorbance is then used in the calculation.

5.5 CALCULATION AND RESULTS

CO₂ mmol/L =

$$\frac{(A1 - A2) - (Ab1 - Ab2)}{(As1 - As2) - (Ab1 - Ab2)} \times \text{concentration of standard (or calibrator)}$$

Example:

$$\text{CO}_2 = \frac{0.120}{0.100} \times 30 \text{ mmol/L} = 36 \text{ mmol/L}$$

with (A1 - A2) - (Ab1 - Ab2) = 0.120
(As1 - As2) - (Ab1 - Ab2) = 0.100
concentration of standard = 30 mmol/L

6.0 INTERPRETATION OF RESULTS

6.1 EXPECTED VALUES (10.9)

The range of expected values is:

23-34 mmol/L

These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

6.2 LIMITATIONS OF PROCEDURE

A number of conditions and substances have been reported to affect serum Carbon Dioxide levels. (10.10, 10.11, 10.12)

6.3 LINEARITY

This test is linear to 40 mmol/L. Samples exceeding the linearity limit should be diluted 1:1 with saline, reassayed and the result multiplied by 2.

7.0 QUALITY CONTROL

Standard practice for quality control should be applied to this system. Commercially available lyophilized controls can be used to monitor the daily acceptable variations. Normal and abnormal controls should be assayed at the beginning of each run of patient samples, whenever a new reagent or a different lot number is being used, and following any system maintenance. A satisfactory level of performance is achieved when the analyte values obtained are within the "acceptable range" established by the laboratory.

8.0 CALIBRATION PROCEDURES

Use an aqueous CO₂ standard or a serum based calibrator. The assay is calibrated by referencing the absorbance of the unknown sample to the absorbance of the standard (or calibrator). Refer to your instrument manual for more details.

Calibration is required with the use of a new lot of reagent, any system maintenance or whenever indicated by quality control data.

9.0 PERFORMANCE CHARACTERISTICS

9.1 PRECISION

Within-Run		
Mean (mmol/L)	SD (mmol/L)	CV (%)
20.7	0.48	2.3
42.7	0.56	1.3

Between-Run		
Mean (mmol/L)	SD (mmol/L)	CV (%)
20.1	0.54	2.7
39.7	1.56	3.9

9.2 CORRELATION

A correlation study was done comparing this method (y) and a commercial reagent using the same methodology (x).

Number of Samples	Regression Equation y=Biotron, x=Comparative	Correlation Coefficient
59	y = 1.10x - 2.4	0.987

10.0 REFERENCES

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