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CARBON DIOXIDE (CO_2) REAGENT SET

INTENDED USE
For manual or automated quantitative determination of Carbon Dioxide content in human plasma or serum at 340 nm.

INTRODUCTION
Carbon Dioxide (CO_2) in serum or plasma exists primarily as dissolved CO_2 and bicarbonate anion (HCO_3^-).[1] The plasma CO_2 content is decreased in metabolic acidosis and respiratory alkalosis, whereas the level is increased in metabolic alkalosis and respiratory acidosis.[2] In pathologic conditions such as in diabetes mellitus, glomerulonephritis, pyloric obstruction, diarrhea, etc., acidosis or alkalosis could be anticipated.[3] Therefore, determination of plasma CO_2 content as part of an electrolyte profile can help establish to a degree, the anticipated change in the patient.

Plasma CO_2 content can be measured manometrically or using an ion selective electrode or by spectrophotometric procedures.[4] This CO_2 reagent measures CO_2 content enzymatically and the procedure is a modification of the method of Forrester et al.[5]

PRINCIPLES OF THE PROCEDURE

$$\text{Phosphoenol Pyruvate} + HCO_3^- \xrightarrow{\text{PEPC}} \text{Oxalate} + H_2PO_4$$

$$\text{Oxalate} + \text{NADH} \xrightarrow{\text{MDH}} \text{Malate} + \text{NAD}$$

Phosphoenol pyruvate carboxylase (PEPC) catalyzes the reaction between phosphoenol pyruvate and carbon dioxide (bicarbonate) to form oxalacetate and phosphate ion. Oxalacetate is reduced to malate with simultaneous oxidation of an equimolar amount of reduced nicotinamide adenine dinucleotide (NADH) to NAD; the reaction is catalyzed by malate dehydrogenase (MDH). This results in a decrease in absorbance at 340 nm that is directly proportional to CO_2 concentration in the sample.

REAGENT COMPOSITION
1. When reconstituted according to the direction, CO_2 reagent contains: PEP 1.8 mM, Magnesium Sulfate 10 mM, NADH 0.40 mM, MDH (porcine) 1,250 U/L, PEPC (microbial) 200 U/L, Sodium Oxamate 2.5 mM, Buffer, (pH 7.0), Non-reactive fillers and stabilizers with Sodium Azide 0.1% as preservative.
2. CO_2 Standard contains 30 mmol/L of Sodium bicarbonate in an aqueous solution.

WARNINGS AND PRECAUTIONS
1. CO_2 reagent is for "In Vitro Diagnostic Use."
2. Normal precautions exercised in handling laboratory reagents should be followed.
3. CO_2 reagent contains sodium azide that is toxic if ingested and which may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide accumulation.
4. Use only CO_2 -free water for reconstitution.
5. Do not pipette by mouth to avoid CO_2 contamination from the expired air.

REAGENT PREPARATION
Reconstitute CO_2 Reagent with the volume of CO_2-free water, indicated on the vial label. Mix by inversion 5-6 times. Do not shake. CO_2 standard is ready to use.

Note: Avoid contamination of reagents with CO_2. Do not blow into pipette, since breath contains a high content of CO_2. Do not let bottles open unnecessarily, since CO_2 from air can contaminate reagent. Keep container tightly stoppered.

STORAGE AND STABILITY
1. Store the unopened vial of CO_2 Reagent and CO_2 standard refrigerated (2 - 8°C). Reagent and standard are stable until the expiration date shown on the labels.
2. Reconstituted reagents stored in the closed tightly stoppered bottle are stable for 1 day at room temperature (18 - 26° C) and 7 days refrigerated (2 - 8° C).

REAGENT DETERIORATION
Do not use the reagent if the absorbance of CO_2 reagent when measure against water in a 1-cm light path is less than 1.000 at 340 nm, or dry reagent exhibit caking due to possible moisture penetration or do not dissolve completely.

SPECIMEN COLLECTION AND STORAGE
1. It is recommended that specimen collection be carried out in accordance with NCCLS document M29-T
2. Serum or heparinized plasma may be assayed. Ideally, venous blood should be collected and handled anaerobically.
3. Plasma and serum, after prompt separation from cells or clot, should be kept tightly stoppered.
4. CO_2 content of blood is stable for 1 hour when stored at 2-4°C under anaerobic conditions.[3,6] Alkalinized serum can stand, without loss of CO_2, for as long as 4 hours in open cups.[7]

INTERFERING SUBSTANCES
The major interference in this assay is CO_2 from air or from the breath of the analyst. Certain drugs and other substances are also known to influence blood CO_2 levels. See Young DS.[8]

MATERIALS PROVIDED
Carbon Dioxide Reagent and Carbon Dioxide Standard.

MATERIALS REQUIRED, BUT NOT PROVIDED
Spectrophotometer, capable of accurately measuring absorbances at 340 nm; Cuvettes with optical properties suitable for use at 340 nm; Pipetting devices for the accurate delivery of volumes required for this assay; Timer; Incubator or water bath, capable of maintaining 37°C.

PROCEDURE
1. Prepare CO_2 Reagent according to REAGENT PREPARATION.
2. Label tubes "Blank", "Standard", "Controls", "Patients", etc.
3. Pipette 1.0 mL Carbon Dioxide Reagent into each tube.
4. Incubate all tubes for 3 minutes at 37°C.
5. Set spectrophotometer wavelength at 340 nm, temperature to 37°C.

6. Pipette 5 µL (0.005 mL) of water, standard, and sample to the cuvette labeled "Blank", "Standard" and "Patients", respectively.
7. Mix gently by inversion and incubate for 5 minutes.
8. Read and record absorbance (Abs.) of all cuvettes at 340 nm.

PROCEDURE NOTES

If the samples is lipemic, icteric, or hemolyzed, a sample blank must be prepared by adding 5 µl of sample to 1.0 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 calculations.

QUALITY CONTROL

The integrity of the reaction should be monitored by use of normal and abnormal controls with known CO_2 values for each assay.

CALIBRATION

CO_2 Standard provided is standardized for using in manual procedure calculation. The concentration of CO_2 should be validated by comparison with commercially available standard. For automated instrument, refer to appropriate application specification.

CALCULATIONS

Determine CO_2 content of sample as follows:

$$CO_2 \text{ Content of Sample (mmol/L)} = \frac{\text{Abs. blank-Abs. sample}}{\text{Abs. blank-Abs. standard}} \times \text{concentration of standard}$$

Example:

Abs. sample = 0.95
Abs. standard = 0.82
Abs. blank = 1.66
Concentration of Standard = 30 mmol/L

$$CO_2 \text{ (mmol/L)} = \frac{1.66 - 0.95}{1.66 - 0.82} \times 30$$
$$= 25.4 \text{ mmol/L}$$

PROCEDURAL LIMITATIONS

CO_2 reagent is linear to 40 mmol/L. If CO_2 content is greater than 40 mmol/L, dilute sample with an equal volume of CO_2-free isotonic saline and reassay. Multiply the result by 2 to compensate for dilution. Lipemic, icteric, or hemolyzed sample will influence the result. Sample blank must be prepared. Please refer to PROCEDURE NOTES section for the preparation of sample blank.

EXPECTED VALUES

23-34 mmol/L.[6]
It is strongly recommended that each laboratory establish an expected range characteristic for the local population.

PERFORMANCE

1. **Linearity:** 0-40 mmol/L.
2. **Sensitivity:** Based on an instrument resolution of Abs.= 0.001, this procedure has a sensitivity of 0.03 mmol/L.
3. **Comparison:**
   Manual procedure: 40 serum samples with CO_2 range from 7.0-30.4 mmol/L were assayed. The study against similar method yielded the correlation coefficient of 0.987 and linear regression equation y = 1.02x - 0.42
   Automated procedure: 70 serum samples with CO_2 range from 0.7-29.7 mmol/L were assayed. The study against similar method yielded the correlation coefficient of 0.99 and linear regression equation y = 1.03x - 0.69
4. **Precision:** The following studies were performed according to NCCLS guideline #EP5-T. Each control was subjected to 20 replicated assays for each study.

Within Run	Control	Mean (mmol/l)	S.D. (mmol/l)	C.V. (%)
Manual procedure	I	34.0	0.6	1.8
	II	16	0.7	4.1
Automated procedure	III	23.13	0.50	2.2
	IV	15.67	0.47	3.0

Run to Run	Control	Mean (mmol/l)	S.D. (mmol/l)	C.V. (%)
Manual procedure	I	33.4	1.4	4.3
	II	15.7	0.7	4.4
Automated procedure	III	23.29	2.00	8.6
	IV	14.79	1.60	10.8

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