POINTE SCIENTIFIC, INC.
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Carbon Dioxide (340nm) Reagent Set

Intended Use
For the quantitative determination of Carbon Dioxide in serum. 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 based on their work.

Principle
$$PEP + HCO_3^- \xrightarrow{PEPC} \text{Oxaloacetate} + H_2PO_4^-$$

$$\text{Oxaloacetate} + NADH + H^+ \xrightarrow{MDH} \text{Malate} + NAD^+$$

Carbon Dioxide (in the form of bicarbonate ions) reacts with phosphoenolpyruvate (PEP), in the presence of phosphoenolpyruvate 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 CO_2 in the sample. Interference from endogenous pyruvate and LDH is eliminated by the inclusion of sodium oxamate.

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
1. CO_2 reagent (concentrations refer to reconstituted reagent): PEP 1.8mM, Magnesium Ions 10mM, NADH 0.40mM, MDH (porcine) ≥ 1200U/L, PEPC (wheat germ) ≥ 200U/L, Sodium Oxamate 2.5mM, Buffer 50mM, pH 8.0 ± 0.1 non-reactive fillers and stabilizers with Sodium Azide (0.1%) as preservative.
2. CO_2 diluent: Reagent diluent with resin to prevent uptake of atmospheric CO_2.

Reagent Preparation
Reconstitute the reagent with the volume of diluent indicated on the vial label. Swirl **gently** to dissolve.

Reagent Storage
1. Unreconstituted reagent should be stored at 2-8°C and is stable until the expiration date on the label.
2. 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 of the reagent.

Reagent Deterioration
Do not use if:
1. There is evidence that moisture has entered the vial, such as caking or incomplete dissolution.
2. The absorbance of the reagent is less than 0.700 at 340nm.

Precautions
1. Reagent contains Sodium Azide at 0.1%. This may react with copper or lead plumbing to form explosive metal azides. Upon disposal, flush with a large volume of water to prevent azide build up.
2. Reagents are for *in vitro* diagnostic use only.
3. Do not ingest. Toxicity has not been established.
4. Use only CO_2 diluent for reconstitution. See "Reagent Preparation".
5. 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. 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.
3. The sample may be stored in ice water under anaerobic conditions for up to one hour.[9]

Interferences
1. CO_2 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.
2. 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. Accurate pipetting devices.
2. Timer/Test tubes/rack
3. Spectrophotometer with ability to read 340 nm.
4. Heating block or water bath (37°C).

Procedure
1. Label test tubes: "Blank", "Standard", "Control", "Patient", etc.
2. Zero spectrophotometer at 340 nm with water.
3. Pipette 1.0ml Carbon Dioxide reagent into each tube and incubate at 37°C for five (5) minutes.
4. Pipette 0.01ml (10ul) standard/calibrator. Mix well.
5. Incubate for 45 seconds at 37°C and take a reading at 340nm.
6. After exactly 30 seconds take a second reading at 340nm.
7. Repeat for each control and patient specimen.
8. Determine the change in absorbance for each sample by subtracting the second reading from the first.
9. To obtain values in mmol/L, see Calculations.

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Procedure Notes
If the result exceeds 40 mmol/L the sample should be diluted 1:1 with saline, re-assayed, and the results multiplied by two.

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.

Calibration
Use an aqueous CO_2 standard (30 mmol/L) or an appropriate serum calibrator.

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

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, control sera with known Carbon Dioxide values should be run with patient samples.

Expected Values [9]
23-34 mmol/L
It is strongly recommended that each laboratory determine its own reference range.

Performance
1. Assay Range: 5 - 40 mmol/L
2. Comparison: A comparison study against another commercial reagent using the same methodology yielded a correlation coefficient of 0.987 and a linear regression equation of y=1.10x – 2.4. (N=59)
3. Precision:

Within Run			Run to Run		
Mean	**S.D.**	**C.V.%**	**Mean**	**S.D.**	**C.V.%**
20.7	0.48	2.3	20.1	0.54	2.7
42.7	0.56	1.3	39.7	1.56	3.9

4. Sensitivity: Following the procedure described, 1 mmol/L Carbon Dioxide will produce an absorbance change of approximately 0.004.

References

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