CARBON DIOXIDE(Bicarbonate)
C and V 206 series (Enzyme cycling)
Manual/Automated Applications

Intended Use
For the quantitative determination of Carbon Dioxide (Bicarbonate) in serum or plasma using a manual application.

Clinical Significance
Determinations of Bicarbonate are primarily used for diagnosing respiratory acidosis and metabolic alkalosis, as well as for monitoring the causes and treatments. (1)

Method History
In 1921 Van Slyke and Stadie (2) published a method for the determination of gases in blood. With availability of phosphoenolpyruvate carboxylase (PEPC), an enzyme which utilizes Bicarbonate as a substrate, simpler and specific methods have been reported. (3, 4) The Catachem Carbon Dioxide (Bicarbonate) procedure for manual or automated applications utilizes a PEPC extracted from a specific microorganism and an enzyme cycling system to prevent contamination by CO2 from the air.

Method Principle
In the presence of bicarbonate, PEPC catalyzes the carboxylation of PEP to form oxaloacetate. Oxalacetate is then quantitated via the MDH-Acetyl NADH reaction with concomitant oxidation of Acetyl NADH to Acetyl NAD. The decrease of the Acetyl NADH absorbance is monitored at 380nm and it is directly proportional to the concentration of bicarbonate in the sample. The reaction scheme illustrates the reactions that occur in this method.

$$\text{Phosphoenolpyruvate} + HCO_3^- \xrightarrow{\text{PEPC}} \text{Oxaloacetate} + \text{Pi}$$

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

Reagent
The reagent comes as a stable liquid the concentrations of the active ingredients in the reagents will be approximately as follows:

Carbon Dioxide Reagent
Each liter contains:

Buffer	
Phosphoenolpyruvate	3.5 mmol
Acetyl NADH	0.6 mmol
PEPC (Microbial)	$\geq$ 600 units
MDH (Microbial)	$\geq$ 750 units
Non reactive ingredients	

Precautions
Avoid contact of reagents with skin and eyes. Should contact occur, wash affected area with plenty of water. **DO NOT PIPETTE REAGENTS BY MOUTH.**

Reagent is provided ready to use.

Reagent Storage And Stability
Store Catachem $C0_2$ Reagent at 2-8˚C. When stored unopened as directed, the reagent is stable until the expiration date stated on the label. Once opened the reagent is stable for at least 6**0 days** or until the baseline OD at 380 nm is less than < 0.5 OD units. After opening when not in use, it is recommended to store the reagent in a **tightly capped** container.

Specimen Collection And Preparation
Serum samples that have been stored at room temperature and exposed to ambient air are not recommended since with time, a significant amount of $C0_2$ will be lost. Samples stored at 2-8˚C and tightly sealed are stable for approximately one hour. (4)

Quality Control
To monitor the performance of the Working Reagents and the procedure used, we recommend the regular use of a normal and abnormal control serum.

Interfering Substances
Several substances have been reported to interfere with the $C0_2$ method. A summary of interference from drugs on clinical laboratory procedures may be found by consulting D.S. Young, et al. (5)

Expected Values
The range of expected values for humans determined for this method is 22 mEq/L to 28mEq/L. Ranges for animals vary by species. All values are considered suggested guidelines. It is recommended that each laboratory establish the normal range for the species under assay and for the area in which it is located. (4)

Procedure
Important: Read entire procedure instructions before proceeding with assay.

Materials Required (Not Provided)

Spectrophotometer	
Match cuvettes	1 cm light path
Timer	to time incubation time
Pipette	1.0 ml for reagent
Pipette	0.01 ml for sample
Calibrator	Catachem Catacal or equivalent calibrator

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Material Provided
Catachem $C0_2$ Reagent

Analytical Parameters

Wavelength	380 nm
Pathlength	1 cm
Reaction mode	endpoint
Reaction time	5.0 minute
Reagent volume	1.0 ml
Sample volume	0.010 ml
Total volume	1.01 ml
Sample to reagent ratio	1:101

Assay Procedures
1. Pipette 1.0 ml of $C0_2$ reagent into each of three cuvettes marked "Calibrator", "Sample", and "Blank".
2. Pipette 0.01 ml of Calibrator or Sample into their respective cuvettes. Use 0.01 ml of distilled water for the Blank. Mix all cuvettes well.
3. Incubate all cuvettes for 5.0 minutes at 37˚C.
4. Set spectrophotometer wavelength at 380nm and zero the instrument with the Blank.
5. Read the "Calibrator" and "Sample" absorbencies.
6. Calculate the $C0_2$ concentration (meq/L) in the Sample(s) as shown in calculations and results.

Calculations and Results *(note Δ means "change in".)*

$$\text{Bicarbonate (meq/L)} = \frac{\text{Sample } \Delta \text{ OD}}{\text{Calibrator } \Delta \text{ OD}} \times \text{Calibrator (meq/L)}$$

Example:

Sample Δ OD	0.300
Calibrator Δ OD	0.200
Calibrator =	20 meq/L

$$\text{Bicarbonate (meq/L)} = \frac{0.30}{0.20} \times 20 \text{ meq/L}$$

$$= 30 \text{ meq/L}$$

Method Performance Characteristics
Sensitivity: The sensitivity of this method is 0.023-0.027 absorbance units/meq/L.
Linear Range: In this method there is no significant nonlinearity over the range of 0-45 meq/L.
Precision: Within-run and day-to-day precision is summarized below:

Precision Study

CO_2	Within-Run		Total Precision	
Mean	SD	CV	SD	CV
Meq/L	Meq/L	%	Meq/L	%
16.0	0.11	0.71	0.15	0.96
23.5	0.27	1.15	0.50	2.14
29.8	0.21	0.70	0.49	1.67

Correlation
A comparison of this method using an automated analyzer and a reference method based upon the PEPC reaction resulted in the following regression statistics.

Range	=	9-36 meq/L
N	=	122
Y	=	1.05x + 0.38
r	=	0.977
Sy.x	=	1.23

References

1. Fundamentals of Clinical Chemistry. Edited by Norbert Teitz. WB Saunders, Philadelphia (1976).
2. Van Slyke DD and Stadie WC. J. Biol. Chem. 49.1 (1921).
3. Menson RC, Narayanswamy V, Bussian RW and Adams TH. Clin Chem 20, 872 (1972).
4. Tietz NW.Textbook of Clin Chem. WB Saunders Co. pp1188, 1209-1210 (1986).
5. Young GS, Pestaner LC and Gibberman V. Effect of drugs on clinical laboratory tests. Clin Chem 21 1D (1975).

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