### 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 CO₂ 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 CO₂.

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 CO₂ have been introduced by Wilson, 6 Menson 7 and Norris 8 using phosphoenolpyruvate carboxylase. The present procedure is a enzymatic assay utilizing Phosphoenolpyruvate Carboxylase (PEPC) and a NADH analog.

### Principle

\[
\text{PEPC} + Mg^{++} + HCO_3^- \rightarrow \text{Oxaloacetate} + H_2PO_4^- + \text{MDH}
\]

\[
\text{Oxaloacetate} + \text{reduced cofactor} + H^+ \rightarrow \text{Malate} + \text{cofactor}
\]

Carbon Dioxide (in the form of bicarbonate ions) reacts with phosphoenolpyruvate (PEP), in the presence of phosphoenolpyruvate carboxylase (PEPC), to form oxaloacetate. The cofactor then in the presence of malate dehydrogenase (MDH) is oxidized by the oxaloacetate. The decrease in absorbance monitored between 405 and 415 nm resulting is proportional to the amount of CO₂ in the sample.

### Clinical Significance
The measurement of Carbon Dioxide 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.

### Reagents
CO₂ reagent: PEP 6mM, Magnesium Ions 10mM, NADH analog, MDH (porcine) ≥ 1200U/L, PEPC (microbial) ≥ 200U/L. Buffer, pH 7.4 ± 0.1 non-reactive stabilizers with surfactants and preservative.

### Reagent Preparation
Reagent provided as a ready to use liquid.

### Reagent Storage
Reagent is stable until expiration date indicated on vial label when stored tightly capped at 2-8°C. (15 months from date of manufacture)

### Reagent Deterioration
1. Reagent should appear clear and pale yellow in color.
2. Do not use if reagent appears to be turbid, this would indicate deterioration.

### Precautions
1. Reagents are for *in vitro* diagnostic use only.
2. Do not ingest. Toxicity has not been established.
3. Do not pipet by mouth to avoid CO₂ contamination from the expired air.

### Specimen Collection and Storage
1. Fresh, unhemolyzed serum collected under anaerobic conditions is the recommended specimen.
2. The sample may be stored in ice water under anaerobic conditions for up to one hour. 9

### Interferences
1. Interferences were evaluated for this carbon dioxide method on a Hitachi 917 analyzer. No interference was observed by bilirubin up to 20.0 mg/dl, hemoglobin up to 400 mg/dl and lipemia (intralipid) up to 1000 mg/dl. (Using a criteria of >10% variance from control. CO₂ level was 19mmol/L)
2. CO₂ from air or the breath of the analyst is a major interference in this assay. Reagent handling, specimen collection, and all storage instructions must be strictly followed to minimize this interference.
3. 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. Spectrophotometer with ability to read between 405 and 415 nm.
2. An aqueous CO₂ standard (30 mmol/L) or an appropriate serum calibrator.
3. To monitor the reliability of results, two levels of control sera with known Carbon Dioxide values.
4. Saline, if samples with a level greater than 40 mmol/L are encountered.

### Procedure (Automated - General)
- **Wavelength:** 405 nm
- **Assay Type:** Fixed Rate
- **Sample/Reagent Ratio:** 1:100
- **Reaction Direction:** Decreasing
- **Lag Phase:** 30 seconds
- **Reaction Time:** 8 minutes

Application parameters for various automated instruments are available. Please contact our Technical Service Department for specific information.

### Procedure (Automated - Hitachi 917)

<table>
<thead>
<tr>
<th>TEST NAME</th>
<th>[Carbon Dioxide ]</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASSAY/ POINT</td>
<td>[2-POINT END] [10] [2] [7] [0] [0]</td>
</tr>
<tr>
<td>WAVE (SUB/MAIN)</td>
<td>[505] [415]</td>
</tr>
<tr>
<td>S. VOL. (NORMAL)</td>
<td>[2.0] [0.0] [0]</td>
</tr>
<tr>
<td>S. VOL. (DECREASE)</td>
<td>[2.0] [0.0] [0]</td>
</tr>
<tr>
<td>S. VOL. (INCREASE)</td>
<td>[2.5] [0.0] [0]</td>
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<tr>
<td>DILUENT</td>
<td>[water] [0]</td>
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<tr>
<td>REAGENT VOL (R1)</td>
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<tr>
<td>REAGENT VOL (R2)</td>
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<td>REAGENT VOL (R3)</td>
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<td>REAGENT VOL (R4)</td>
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<td>ABS. LIMIT</td>
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<tr>
<td>PROZONE LIMIT:</td>
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<tr>
<td>CELL DETEGERNT</td>
<td>[DETERGENT 1]</td>
</tr>
</tbody>
</table>

| CALIB TYPE | [LINEAR] [1] |
| POINT | [2] [SPAN POINT] [2] |
| WEIGHT | [0] |
Carbon Dioxide
(Liquid)
Reagent Set

It is strongly recommended that each laboratory determine its own reference range.

Performance
1. Assay Range: 2 - 40 mmol/L
2. Low Limit of Detection: 2 mmol/L
3. Comparison: A comparison study against another commercial reagent using the same methodology yielded a correlation coefficient of 0.986 and a linear regression equation of \( y = 0.965x + 1.2 \). (N=136, Std Err Est=1.1)
4. Precision: Within Day precision was investigated by running two samples in replicates of 20 on the same day. Day to Day results were obtained by performing one run per day over a span of 20 days. These studies were performed on a Roche Diagnostics, Hitachi 917 chemistry analyzer.

Within Day (n=20) Day to Day (n=20)

<table>
<thead>
<tr>
<th>Mean</th>
<th>S.D.</th>
<th>C.V.%</th>
<th>Mean</th>
<th>S.D.</th>
<th>C.V.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.5</td>
<td>0.5</td>
<td>3.1</td>
<td>16.2</td>
<td>0.5</td>
<td>2.9</td>
</tr>
<tr>
<td>27.4</td>
<td>0.6</td>
<td>2.2</td>
<td>27.1</td>
<td>0.9</td>
<td>3.1</td>
</tr>
</tbody>
</table>

References

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. Keep reagent tightly capped when not in use.

Calibration
Use an aqueous CO₂ standard (30 mmol/L) or an appropriate serum calibrator.

Calculation
\( \text{Abs. Sample} \times \text{C}_\text{S} = \text{Carbon Dioxide} \)
\( \text{Abs. Standard} \)

Where \( \text{C}_\text{S} \) = 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:

0.225 \times 30 \text{ mmol/L} = 27 \text{ mmol/L}
0.250

Quality Control
To monitor the reliability of results, two levels of control sera with known Carbon Dioxide values should be run with patient samples.

Expected Values
23-34 mmol/L

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