Carbon Dioxide (CO$_2$)

Clinical Significance:

Elevated blood CO$_2$ is almost synonymous with respiratory acidosis. The latter is restricted to clinical conditions with a primary increase in CO$_2$ in the inspired air or increased metabolic production of CO$_2$. Decreased blood CO$_2$ is almost synonymous with respiratory alkalosis. The later is restricted to clinical conditions with a primary decrease in CO$_2$ which can result from increased pulmonary ventilation due to mechanical ventilation or stimulation of the respiratory center.

Classic techniques for the measurement of CO$_2$ involve the addition of acid to liberate the CO$_2$ and the measurement of CO$_2$ thus released by either manometric, volumetric, titrimetric techniques. These procedures are both time consuming and cumbersome. This CO$_2$ assay is an enzymatic procedure, employing phosphoenolpyruvate carboxylase (PEPC) and a stabilized NADH which is easy to use and applicable to routine laboratory instrumentation.

**Principle:**

\[
\text{PEPC} \quad \text{PEP} + \text{HCO}_3^- \rightarrow \text{Oxaloacetate} + \text{H}_2\text{PO}_4^- \\
\text{Oxaloacetate} + \text{NADH} + \text{H}^+ \rightarrow \text{Malate} + \text{NAD}
\]

PEPC catalyses the first reaction which produces oxaloacetate. In the presence of MDH, the reduced cofactor is oxidized by oxaloacetate. The decrease in concentration of the reduced cofactor is monitored between 405 and 415 nm and is proportional to the total CO$_2$ concentration in the sample.

**Reagents:**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>PEP</td>
<td>12.5mmol/L</td>
</tr>
<tr>
<td></td>
<td>MgCl$_2$</td>
<td>8.0mmol/L</td>
</tr>
<tr>
<td></td>
<td>PEPC</td>
<td>400U/L</td>
</tr>
<tr>
<td></td>
<td>MDH</td>
<td>4100U/L</td>
</tr>
<tr>
<td></td>
<td>COFACTOR</td>
<td>0.6mmol/L</td>
</tr>
<tr>
<td>Standard</td>
<td>NaHCO$_3$</td>
<td></td>
</tr>
</tbody>
</table>

**Precautions:**

Avoid contact with skin and eyes.

**Calibration:**

Calibration material should be used to calibrate the procedure. The frequency of calibration, if necessary using an automated system is dependent on the system and the parameters used.

**Reagent deterioration:**

The presence of particles and turbidity.

**Storage and Stability:**

Reagents are ready to use. Supplied reagent stable at 2-8°C until expiry date.

**Materials required:**

- Thermostatic bath at 37°C.
- Spectrophotometer or photometer thermostable at 37°C with a 405 nm filter (405 – 420 nm).

**Specimen and Stability:**

Freshly drawn serum or plasma are the specimens of choice. Serum or plasma should be separated from cells immediately and stored at 2-8°C.

Exposure of samples to air should be minimized. Samples should be stored tightly sealed to prevent loss of CO$_2$ and assayed as soon as possible after collection. For plasma samples, the anticoagulant, lithium heparin, has been tested and may be used with this assay.

**Assay Procedure:**

1. **Assay conditions:**
   - Wavelength: 405 nm (405-420)
   - Cuvette: 1 cm light path
   - Constant temperature: 37°C

2. **Procedure:**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>10µl</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>10µl</td>
<td>-</td>
</tr>
</tbody>
</table>

3. Mix. Incubate for 8 minutes at 37°C. Read the absorbance and record A of the standard and sample

**Calculations:**

\[
\text{CO}_2 = \frac{A \text{ Sample}}{A \text{ Standard}} \times \text{Standard value}
\]

**Quality Control:**

Commercially available normal and pathological control sera are recommended to monitor the performance of the procedure.

If the controls are found outside the defined range, check the instrument, reagents and calibrator for problems.

| Serum controls are recommended for internal Quality control. Each laboratory should establish its own Quality Control scheme and corrective actions |

**Reference Values:**

23mmol/L – 29mmol/L (23-29 mEq/L)

These values are for orientation purpose. Each laboratory should establish its own reference range.

**Performance Characteristics:**

**Linearity**

The method is linear up to 50mmol/L. Samples above this concentration should be diluted 1+1 with 0.9% NaCl solution and the result multiplied by 2.

**Analytical sensitivity (Low detection limit)**

Detection limit: 1 mmol/L.
Carbon Dioxide (CO₂)

Precision

Reproducibility was determined using human samples or controls in an internal protocol (n = 21). The following results were obtained.

Within Run Precision

<table>
<thead>
<tr>
<th>Level</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (mmol/L)</td>
<td>15</td>
<td>18</td>
<td>25</td>
</tr>
<tr>
<td>SD</td>
<td>0.26</td>
<td>0.28</td>
<td>0.3</td>
</tr>
<tr>
<td>CV (×)</td>
<td>1.73%</td>
<td>1.56%</td>
<td>1.20%</td>
</tr>
</tbody>
</table>

Between Run Precision

<table>
<thead>
<tr>
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</tr>
<tr>
<td>SD</td>
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<td>0.32</td>
</tr>
<tr>
<td>CV (×)</td>
<td>1.94%</td>
<td>1.28%</td>
</tr>
</tbody>
</table>

Interferences:
1. Lipemia (Intralipid): no interference up to 1000 mg/dl of intralipid.
2. Hemolysis: no interference up to 1000 mg/dl of hemoglobin.
3. Vc: no interference up to 40 mg/dl of Vc.
4. Bilirubin: no interference up to 40 mg/dl.

The result may vary with different analyzers or calibrations.

Test parameters:

Mode : End point
Reaction : Descending
Wavelength : 405 nm (400nm ~ 420nm)
Sample Volume : 10 µl
Reagent Volume : 1000 µl
Incubation : 8 mins
Calibrator : See vial label
Normal range : 23 mmol/L ~ 29 mmol/L
Linearity limit : 50 mmol/L
Unit : mmol/L

Notes:

Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

Literature:

Manufactured by: Biosino Bio-Technology and Science Inc., P.R.C
Marketed By: Euro Diagnostic Systems Pvt. Ltd., Millennium House, M.K.Srinivasan Nagar Main Road, No. 144, Old Mahabalipuram Road, Perungudi, Chennai – 600 096; Email: eurods@vsnl.net, www.eurods.in