CLINICAL SIGNIFICANCE  
Total carbon dioxide content of plasma consists of CO₂ dissolved in an aqueous solution, CO₂ loosely bound to amine groups in proteins (carbamino compounds), bicarbonate HCO₃⁻, vanishingly small amounts of CO₃²⁻ ions and carbonic acid (H₂CO₃). Approximately 90% of total carbon dioxide in plasma is in form of bicarbonate.
Measurement of total CO₂ as a part of an electrolyte profile (Na⁺, K⁺, Cl⁻), and with blood gases and pH values, is useful chiefly to evaluate HCO₃⁻ concentration in assessment of acid-base disorders resulting from metabolic or respiratory causes.

PRINCIPLE  
Enzymatic methods for determining total CO₂ as bicarbonate and dissolved gases. Reaction scheme is as follows:

HCO₃⁻ + Phosphoenopyruvate → Oxaloacetate + H₂PO₄⁻

Oxaloacetate + NADH → Malate + NAD⁺

The decrease in absorbance due to the oxidation of NADH in NAD⁺ is directly proportional to the amount of total CO₂ in the specimen and is measured at 380 nm.

REAGENTS COMPOSITION

<table>
<thead>
<tr>
<th>Vial R1</th>
<th>WORKING REAGENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphoenopyruvate</td>
<td>8.0 mmol/L</td>
</tr>
<tr>
<td>NADH</td>
<td>1.6 mmol/L</td>
</tr>
<tr>
<td>PEPC (Phosphoenolpyruvate carboxilase) (Malate dehydrogenase)</td>
<td>&gt; 1000 IU/L</td>
</tr>
<tr>
<td>Buffer pH (20°C)</td>
<td>8.0 ± 0.1</td>
</tr>
<tr>
<td>Stabiliser</td>
<td>66 mmol/L</td>
</tr>
</tbody>
</table>

Before reconstitution: Xn, R22: Harmful if swallowed
S22: Do not breathe dust
S28: after contact with skin, wash immediately with plenty of water
S36: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

Once reconstituted: None

<table>
<thead>
<tr>
<th>Vial R2</th>
<th>STANDARD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium bicarbonate</td>
<td>27 mmol/L</td>
</tr>
</tbody>
</table>

SAFETY CAUTIONS
BIOLABO reagents are designated for professional, in vitro diagnostic use.
- Verify the integrity of the contents before use.
- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with skin or eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
- Reagents contain sodium azide (concentration < 0.1%) which may react with copper and lead plumbing. Flush with plenty of water when disposing.
- Material Safety Data Sheet is available upon request.
- Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

REAGENTS PREPARATION
Add promptly to the contents of vial R1 the amount of distilled or deionised water stated on the label. Mix gently and wait for complete dissolution before using reagent (approximately 2 minutes). To avoid CO₂ contamination, use fresh distilled water, not stored longer than 1 day. Do not pipette by mouth.

STABILITY AND STORAGE
Store away from light, well capped in the original vial at 2-8°C.
- Reagent (vial R1) and Standard (vial R2): Transfer requested quantity, recap and store at 2-8°C.
- Unopened, reagents are stable until expiry date stated on the label of the kit when stored and used as described in the insert.
- Once reconstituted, working reagent is stable for 4 months when free from contamination. Recap promptly after use.
- Discard any reagent if cloudy or if absorbance measured at 380 nm is < 1.000.
- Don’t use working reagent after expiry date stated on the label of the Kit.

SPECIMEN COLLECTION AND HANDLING
Plasma or Serum, venous. Collect specimen anaerobically. Heparin is the preferred anticoagulant.
Do not store specimen more than 1 hour at 2-8°C.

INTERFERENCES

Studies on sera with Cobas Mira (340 nm) show results as follows:

<table>
<thead>
<tr>
<th>Interferent</th>
<th>Bicarbonates in specimen (mmol/L)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>27.9 mmol/L</td>
<td>No interference up to 25 mg/dL</td>
</tr>
<tr>
<td>Total Bilirubin</td>
<td>31.3 mmol/L</td>
<td>No interference up to 500 µmol/L</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>25.6 mmol/L</td>
<td>No interference up to 248 µmol/L</td>
</tr>
<tr>
<td>Glucose</td>
<td>28.6 mmol/L</td>
<td>No interference up to 1000 µg/dL</td>
</tr>
<tr>
<td>Lipemia</td>
<td>26.3 mmol/L</td>
<td>No interference of the turbidity up to 0.283 abs (measured at 600 nm)</td>
</tr>
</tbody>
</table>

Studies on sera with Spectrophotometer (380 nm) show results as follows:

<table>
<thead>
<tr>
<th>Interferent</th>
<th>Bicarbonates in specimen (mmol/L)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>17.2 mmol/L</td>
<td>No interference up to 25 mg/dL</td>
</tr>
<tr>
<td>Total Bilirubin</td>
<td>24.1 mmol/L</td>
<td>No interference up to 500 µmol/L</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>24.2 mmol/L</td>
<td>Positive interference above 130 µmol/L</td>
</tr>
<tr>
<td>Glucose</td>
<td>23.0 mmol/L</td>
<td>No interference up to 1000 mg/dL</td>
</tr>
<tr>
<td>Lipemia</td>
<td>13.6 mmol/L</td>
<td>Interference above 0.050 abs (measured at 600 nm)</td>
</tr>
</tbody>
</table>

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.
**MATERIAL REQUIRED BUT NOT PROVIDED**

1. Basic medical analysis laboratory equipment.
2. Normal and pathological control sera.
3. Demineralised water for the preparation of reagent.

**CALIBRATION**
- Standard (vial R2) enclosed in the kit measured in standardized conditions with enzymatic method and aqueous standard traceable to NERL Standard
- or any calibrator traceable to a reference method or material.

The calibration frequency depends on proper instrument functions and on the preservation of reagent.

It is recommended to calibrate in the following cases:
1. When using a new batch of reagent.
2. After maintenance operations on the instrument.
3. When control values are out of range, even after using a new vial of fresh serum.

**QUALITY CONTROL**
- Normal Control Ethanol Ammonia Bicarbonate
- Pathological Control Ethanol Ammonia Bicarbonate
- External quality control program.

It is recommended to control in the following cases:
1. At least once a run.
2. At least once within 24 hours.
3. When changing vial of reagent.
4. After maintenance operations on the instrument.

**MANUAL PROCEDURE**
Let stand reagent and specimens at room temperature.

1. Repeat the test with the same control.
2. After maintenance operations on the instrument.
3. When using a new batch of reagent.
4. After changing vial of reagent.

**EXPECTED VALUES**

<table>
<thead>
<tr>
<th>Carbon dioxide total (mEq/L)</th>
<th>(mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult:</td>
<td>23-29</td>
</tr>
<tr>
<td>&gt; 60 years:</td>
<td>23-31</td>
</tr>
<tr>
<td>&gt; 90 years:</td>
<td>20-29</td>
</tr>
</tbody>
</table>

Each laboratory should establish its own normal ranges for the population that it serves.

**PERFORMANCES CHARACTERISTICS**

<table>
<thead>
<tr>
<th></th>
<th>Low level</th>
<th>High level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean mEq/L</td>
<td>10.0</td>
<td>40</td>
</tr>
<tr>
<td>S.D. mEq/L</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>C.V. %</td>
<td>7.3</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>Low level</td>
<td>High level</td>
</tr>
<tr>
<td>Mean mEq/L</td>
<td>10.0</td>
<td>40</td>
</tr>
<tr>
<td>S.D. mEq/L</td>
<td>0.8</td>
<td>1.6</td>
</tr>
<tr>
<td>C.V. %</td>
<td>8.0</td>
<td>3.9</td>
</tr>
</tbody>
</table>

Detection limit: approximately 3.0 mEq/L
Sensitivity for 1 mEq/L: approximately 0.010 ΔAbs.

Comparison study with a commercially available reagent (enzymatic method): 67 sera within 15 and 43 mmol/L have been evaluated with both reagents. Results compared by least squares regression are as follows:

\[ y = 0.96x + 0.40 \]

**LINEARITY**
The assay is linear up to 50 mEqL (50 mmol/L).
Above, dilute specimen with demineralised (CO₂-free) water and reassay taking into account dilution factor. Linearity limit depends on specimen/reagent ratio.

**CALCULATION**
Calculate the result as follows:

\[
\text{Total CO}_2 = \frac{(\text{Abs }_\text{Blank} - \text{Abs }_\text{Assay})}{(\text{Abs }_\text{Blank} - \text{Abs }_\text{Standard})} \times \text{Standard concentration}
\]

(*) Total CO₂ is approximately 10 % higher than Bicarbonate.

**REFERENCES**


Made in France Latest revision: www.biolabo.fr Revision: 29/07/2011