**SUMMARY OF TEST**

Bicarbonate is the second largest fraction of the anions in plasma. Total carbon dioxide (CO₂) content of plasma consists of carbon dioxide dissolved in an aqueous solution (CO₂−) and vanishingly small amounts of carbonate (CO₃²⁻) ions, and carbonic acid (H₂CO₃); but at the physiological pH of blood, the concentration of carbonate is 1/1000 that of bicarbonate and the carbonic acid compounds are also present in such low quantities that they are generally not mentioned specifically. Several different methods for the determination of bicarbonate in serum and plasma have been reported. One of the earliest methods for determining total CO₂ was manometric method, using the Natelson microgasometer. These methods were either cumbersome, time-consuming, technique-oriented, and required specialized equipment. This has been supplanted in clinical laboratories by automated methods. The first step in automated methods is acidification or alkalinization of the sample. Acidifying the sample converts all CO₂ and carbonic acid to H₂CO₃, which is then trapped as a gas and measured. The principles of these methods are well established.

**PRINCIPLE OF THE METHOD**

Bicarbonate reacts with phosphoenolpyruvate (PEP), in the presence of phosphoenolpyruvate carboxylase (PEP-C), to form oxaloacetate and phosphate.

\[
\text{PEP} + \text{HCO}_3^- \rightarrow \text{PEP-C} + \text{H}_2\text{PO}_4^-
\]

The oxaloacetate is then converted to malate by the action of malate dehydrogenase (MDH) and reduced nicotine adenine dinucleotide (NADH-analog). Measurement of the total CO₂ as part of an electrolyte profile is useful chiefly to evaluate HCO₃⁻ concentration in assessment of acid-base disorders, infract the bicarbonate content of serum or plasma is a significant indicator of acidosis or respiratory imbalance in the respiratory and metabolic systems.

**MATERIALS REQUIRED BUT NOT SUPPLIED**

BR F060 CH 6 x 10 ml
BR F245 CH 12 x 20 ml
BR F400 CH 4 x 100 ml

**KIT COMPONENTS**

For in vitro diagnostic use only.

The components of the kit are stable until expiration date on labels. It is suggested to handle carefully it, avoiding contact with skin and swallow.

**PREREQUISITE**

Serum, heparin plasma. Specimens should be protected from direct exposure to light. Samples stored at 2-8°C in the dark are stable up to 3 days and 1 month at -20°C.

**TEST PROCEDURE**

| Wavelength: | 415 nm (allowed 400 + 415 nm) |
| Lightpath: | 1 cm |
| Temperature: | 25, 30 or 37°C |

**Dispense:** blank standard sample

| Water | 1 ml | 1 ml | 1 ml |
| Standard | 10 | - | - |
| Sample | - | 10 | - |
| Mix, incubate at 25, 30 or 37°C for 2 minutes. Read absorbances of standard (As) and samples (Ax) against reagent blank. |

**RESULTS CALCULATION**

Bicarbonate mmol/l = \((Ax / As) \times \text{standard value}\)

**EXPECTED VALUES**

- Newborn: 13 - 22 mmol/l - mEq/l
- Infant, child: 20 - 28 mmol/l - mEq/l
- Adults: 22 - 29 mmol/l - mEq/l
- Adults > 60 yr: 23 - 31 mmol/l - mEq/l

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

**QUALITY CONTROL AND CALIBRATION**

It is suggested to perform an internal quality control. For this purpose a suitable human based control sera has to be used.

Please contact Customer Care for further information.

**TEST PERFORMANCE**

**Linearity**

The method is linear up to 50 mmol/l. If the limit value is exceeded, it is suggested to dilute sample 1+1 with distilled water and to repeat the test multiplying the result by 5.

**Sensitivity/limit of detection (LOD)**

The limit of detection is 1.182 mmol/l.

**Interferences**

No interference was observed by the presence of:
- lipids ≤ 700 mg/dl
- bilirubin ≤ 8 mg/dl
- lipids ≤ 700 mg/dl
- HDL ≤ 100 mg/dl
- triglycerides

**Precision**

Methods comparison

<table>
<thead>
<tr>
<th>Microsample</th>
<th>Serum</th>
<th>Hb</th>
<th>Bicarbonate</th>
</tr>
</thead>
<tbody>
<tr>
<td>intra-assay</td>
<td>sample 1</td>
<td>17.70</td>
<td>0.28</td>
</tr>
<tr>
<td>inter-assay</td>
<td>sample 2</td>
<td>30.50</td>
<td>0.56</td>
</tr>
</tbody>
</table>

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**Reagent A**

<table>
<thead>
<tr>
<th>Composition</th>
<th>Buffer 0.1 M, PEP 10 mM, PECC &gt; 300 UI, MDH&gt;1000 UI, NAD-analog 0.5 mM, stabilizer and preservative.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A</td>
<td>F060: 6 x 10 ml (liquid) blue cap</td>
</tr>
<tr>
<td></td>
<td>F245: 12 x 20 ml (liquid) blue cap</td>
</tr>
<tr>
<td></td>
<td>F400: 4 x 100 ml (liquid) blue cap</td>
</tr>
</tbody>
</table>

Composition: Buffer 0.1 M, PEP 10 mM, PECC > 300 UI, MDH>1000 UI, NAD-analog 0.5 mM, stabilizer and preservative.

**Standard**

bicarbonate solution 30 mmol/l - 5 ml

Store all components at 2-8°C.

**MATERIALS REQUIRED BUT NOT SUPPLIED**


**REAGENT PREPARATION**

Use single reagent ready to use. Stability: up to expiration date on labels at 2-8°C.

Stability since first opening of vials: preferably within 60 days at 2-8°C keep away from direct light sources.

**PRECAUTIONS**

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.

Perform the test according to the general “Good Laboratory Practice” (GLP) guidelines.