Indications For Use: For the Quantitative Determination of β-Hydroxybutyrate in Human Serum or Plasma.

Summary and Principle

Ketosis is a common feature in acutely ill patients. In subjects suffering from starvation, acute alcohol abuse, or diabetes mellitus, ketosis can result in severe life threatening metabolic acidosis. The presence and degree of ketosis can be determined by measuring blood levels of β-hydroxybutyrate.

Ordinarily, β-hydroxybutyrate is the ketoacid present in the greatest amount in serum. It accounts for approximately 75% of the ketone bodies which also contain acetoacetate and acetone. During periods of ketosis, β-hydroxybutyrate increases even more than the other two ketoacids, acetoacetate and acetone, and has been shown to be a better index of ketoacidosis including the detection of subclinical ketosis.

In diabetics, the measurement of β-hydroxybutyrate as well as the blood glucose is needed for the assessment of the severity of diabetic coma and is essential for the exclusion of hyperosmolar non-ketotic diabetic coma. Moreover, the insulin requirements are often based on the extent of the existing hyperketonemia shown by the blood levels of β-hydroxybutyrate.

Enzymatic quantitation of β-hydroxybutyrate by β-hydroxybutyrate dehydrogenase has been reported. In the Stanbio method, β-hydroxybutyrate (D-3-hydroxybutyrate) in the presence of NAD gets converted to acetoacetate and NADH at pH 8.5 by β-hydroxybutyrate dehydrogenase (D-3-hydroxybutyrate dehydrogenase). At this pH, the reaction is favored to the right. The NADH produced reacts with INT in the presence of diaphorase to produce color at 505 nm.

Reagents

Enzyme (R1), Cat No. 2441
Contains β-hydroxybutyrate dehydrogenase and diaphorase enzymes.

Catalyst (R2), Cat No. 2442
Contains NAD, INT, and oxalate.

Standard, 1 mmol/L, Cat. No. 2443
Contains 1 mM Sodium D-3-hydroxybutyrate.

Precautions

For In Vitro Diagnostic Use Only. Rx only. Avoid skin contact with the reagents. If this occurs wash immediately with water.

Reagent Preparation:
Reagents are supplied ready to use.

Reagent Storage and Stability:
Reagents are stable stored at 2-8°C until expiration date on their respective labeling. Once opened, contamination must be avoided.

Deterioration:
Reagents should be clear solutions. Discard reagents if turbidity, discreet particles or any changes indicating microbial contamination occur.

Materials Required But Not Provided

Spectrophotometer capable of absorbance readings at 505 nm
Temperature controlled incubator
Accurate pipetting devices, Timer, Cuvettes
Reagent grade water or saline as the zero calibrator or diluent

Specimen Collection and Preparation

Serum or plasma collected with EDTA, heparin, or sodium fluoride can be used in the assay. Avoid hemolysis.

Sample Stability: Serum or plasma β-hydroxybutyrate levels are stable at least one week if kept refrigerated (2-8°C).

Interfering Substances: No significant changes in values were observed when the following analytes were added to serum containing 0.5 mM β-hydroxybutyrate.

<table>
<thead>
<tr>
<th>Substance</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (2000 mg/dL)</td>
<td>96</td>
</tr>
<tr>
<td>Acetoacetic acid (5 mM)</td>
<td>96</td>
</tr>
<tr>
<td>Creatinine (5 mg/dL)</td>
<td>106</td>
</tr>
<tr>
<td>Ascorbate (3 mg/dL)</td>
<td>106</td>
</tr>
<tr>
<td>Bilirubin (10 mg/dL)</td>
<td>96</td>
</tr>
<tr>
<td>Uric Acid (16 mg/dL)</td>
<td>102</td>
</tr>
<tr>
<td>Triglycerides (417 mg/dL)</td>
<td>104</td>
</tr>
<tr>
<td>Cholesterol (314 mg/dL)</td>
<td>94</td>
</tr>
<tr>
<td>Lactic dehydrogenase (1515 U/mL)</td>
<td>93</td>
</tr>
<tr>
<td>Sodium lactate (96 mg/dL)</td>
<td>99</td>
</tr>
</tbody>
</table>

In addition, hemolysed serum with an OD at 540 nm of 2.0 was added to the test and found not to interfere.

Automated Analyzer

Consult the application for Ortho VITROS 5600 Integrated System, VITROS 4600 Chemistry System, and VITROS XT 7600 Integrated System for program parameters.

Quality Control

1) Stanbio Laboratory recommends the use of Stanbio TDM/β-Hydroxybutyrate Tri-Level Controls (Catalog No. 2460-605) or TDM/β-Hydroxybutyrate Bi-Level Controls (Catalog No. 2465-605). See the instructions for use for use and control ranges.

2) Two or three levels of controls are run each day the instrument is run prior to reporting patient results.

3) Refer to the instructions for use for the established ranges. Recovered control values outside of the established ranges are considered out of control and require the following corrective action.

Performance Characteristics for Ortho VITROS 4600

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>≤ 0.02 mmol/L</td>
</tr>
<tr>
<td>Limit of Blank</td>
<td>≤ 0.02 mmol/L</td>
</tr>
<tr>
<td>Limit of Detection</td>
<td>0.016 mmol/L</td>
</tr>
<tr>
<td>Limit of Quantitation</td>
<td>0.031 mmol/L at 10% CV</td>
</tr>
</tbody>
</table>

Linearity

Conducted in accordance with CLSI EP06-A.

0.02 - 6.0 mmol/L
Precision
Conducted in accordance with CLSI EP05-A3 over 22 days.

<table>
<thead>
<tr>
<th></th>
<th>Control Level 1</th>
<th>Control Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within run</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (mmol/L)</td>
<td>0.188</td>
<td>4.232</td>
</tr>
<tr>
<td>Coefficient of variation (%)</td>
<td>1.06</td>
<td>0.57</td>
</tr>
<tr>
<td>Total run</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (mmol/L)</td>
<td>0.188</td>
<td>4.232</td>
</tr>
<tr>
<td>Coefficient of variation (%)</td>
<td>1.06</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Method comparison
105 samples (85 serum/20 plasma) were run on Ortho VITROS 4600 compared to Sirrus analyzer, in accordance with CLSI EP09-A3.
y = 0.97x – 0.0227 with correlation coefficient of 0.9972

Performance Characteristics for Ortho VITROS 5600 System are applicable to the VITROS XT 7600 System

Sensitivity
Conducted in accordance with CLSI EP17-A2.
Limit of Blank ≤ 0.02 mmol/L
Limit of Detection 0.012 mmol/L
Limit of Quantitation 0.031 mmol/L at 10% CV

Linearity
Conducted in accordance with CLSI EP06-A.
0.02 – 8.0 mmol/L

Precision
Conducted in accordance with CLSI EP05-A3 over 22 days.

<table>
<thead>
<tr>
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<th>Control Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within run</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (mmol/L)</td>
<td>0.194</td>
<td>4.209</td>
</tr>
<tr>
<td>Coefficient of variation (%)</td>
<td>2.58</td>
<td>0.67</td>
</tr>
<tr>
<td>Total run</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (mmol/L)</td>
<td>0.194</td>
<td>4.209</td>
</tr>
<tr>
<td>Coefficient of variation (%)</td>
<td>3.09</td>
<td>0.86</td>
</tr>
</tbody>
</table>

A high analyte sample can produce an absorbance within the VITROS System photometer range but above the assay measuring range resulting in an OR code.

A very high analyte sample can produce an absorbance outside the VITROS System photometer range resulting in a CB code.

For either the OR or CB codes, dilute the sample 3X with reagent grade water or saline, and retest. This may be accomplished by off-line manual dilution or an operator requested onboard dilution using either VITROS Chemistry Products FS Diluent Pack 2 (BSA/Saline) or VITROS Chemistry Products FS Diluent Pack 3 (Specialty Diluent/Water). Multiply the final result by 3 if off-line manual dilution is used and the manual dilution factor was not entered while programming the sample.

Find Symbol Glossary at www.ekfusa.com/symbols-glossary

References

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