β-Hydroxybutyrate LiquiColor®

METHOD: β-Hydroxybutyrate Dehydrogenase/INT

SPECIMEN: Serum or Plasma

SPECIMEN COLLECTION AND PREPARATION:

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

SAMPLE STABILITY:

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

ASSAY TEMPERATURE: 37 °C

PRECAUTIONS: *For In Vitro Diagnostic Use Only.*
Avoid skin contact with the reagents. If this occurs wash immediately with water.

CONTROLS: TDM/β-Hydroxybutyrate Tri-Level or Bi-Level Controls
(Cat. 2460-605, 2465-605)

MATERIALS (REAGENTS and TANDARD) PROVIDED:

Reagent A (R1): 50 mL solution containing β-Hydroxybutyrate dehydrogenase and diaphorase enzymes
Reagent B (R2): 8.5 mL solution containing NAD, INT, and Oxalate
Standard: 1.00 mmol/L of β-Hydroxybutyrate
Standard (1 mM): 3 mL solution containing buffer and Sodium-D-3-Hydroxybutyric acid.

MATERIALS REQUIRED BUT NOT PROVIDED:

Spectrophotometer capable of absorbance readings at 505 nm
Temperature controlled incubator
Accurate pipetting devices
Timer
Cuvettes

LIMITATIONS: Lactic dehydrogenase and lactate have been shown to interfere with the assay. The incorporation of oxalic acid in this reagent eliminates this interference as reported.

<u>Interfering Substances</u>
No significant changes in values were observed when the following analytes were added to serum containing 0.5 mM ß-hydroxybutyrate.

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

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

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 ß-hydroxybutyrate.

Ordinarily, ß-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.[2,3,4] During periods of ketosis, ß-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.[5,6,7,8]

In diabetics, the measurement of ß-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[9] shown by the blood levels of ß-hydroxybutyrate is therefore extremely important in the assessment of ketosis.

Enzymatic quantitation of ß-hydroxybutyrate by ß-hydroxybutyrate dehydrogenase has been reported.[10,11,12] In the Stanbio method, ß-hydroxybutyrate (D-3-hydroxybutyrate) in the presence of NAD gets converted to acetoacetate and NADH at pH 8.5 by ß-hydroxybutyrate dehydrogenase (D-3-hydroxybutyrate dehydrogenase). At this pH, the reaction is favored to the right.[12] The NADH produced reacts with INT in the presence of diaphorase to produce color at 505 nm.

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

REAGENT PREPARATION:

Reagents are supplied ready to use.

PROCEDURE:

Manual Method

The ß-Hydroxybutyrate Reagent provided can be used in a five minute procedure utilizing a spectrophotometer which reads absorbance at 505 nm wavelength.

Wavelength.....505 nm
Temperature.....37 °C
Mode.....End Point
Sample Volume.....60 µL
Reagent Volume.....2.51 mL
Sample to Reagent Ratio.....1/42

1) Incubate the needed amount of Reagent A (Enzyme) at 37 °C for 3 minutes.
2) To two 3 mL cuvettes, add 2.15 mL of Reagent A (Cuvettes 1 and 2).
3) To cuvette 1, add 60 µL of sample to be tested and immediately measure the OD at 505nm (To).
4) To the same cuvette 1, add 0.36 mL of Reagent B (Catalyst) and measure the final OD at 505nm (Tf) at 5 minutes.
5) To cuvette 2, add 60 µL of Hydroxybutyrate Standard and immediately measure OD at 505nm (To, std).
6) To the same cuvette 2, add 0.36 mL of Reagent B and measure the final OD at 505nm (Tf, std) at 5 minutes.
7) Subtract To from Tf to obtain OD (5min) for both serum and standard.

Automated Method

Refer to the appropriate operator's manual and/or Application Sheet for analyzer specific assay instructions.

RESULTS:

Calculation

$$\text{ß-Hydroxybutyrate (mM)} = \frac{\text{OD (5 min) Sample x 1mM x dilution of serum}}{\text{OD (5 min) Std}}$$

NOTES:
a) Precise measurement of temperature, wavelength and time are required to obtain accurate results.
b) The test can also be performed at 25 °C for 10 minutes using the same procedure as above.
c) To obtain mg/dL, divide the value obtained in mM by 0.096.

QUALITY CONTROL:

To assure consistent performance, it is recommended that normal and abnormal serum controls be assayed with each run.

EXPECTED VALUES:

The quantitation of ß-hydroxybutyrate is important in cases of ketoacidosis. In studies of healthy individuals who had fasted for 12 hours before blood collection, the range of ß-hydroxybutyrate was found to be from 0.02 mM (0.2 mg/dL) to 0.27 mM (2.81 mg/dL).

REFERENCE: Stanbio β-Hydroxybutyrate LiquiColor® Instruction For Use, RBR.2440CE

Date of Review/Revision:

Reviewed by_____
Lab Director/Supervisor

DN: CLSI.2440.00 rev. 10/2011