ß-Hydroxybutyrate LiquiColor®
Procedure No. 2440

Indications For Use: For the Quantitative Determination of ß-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.[1] 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.

$$\text{D-3-Hydroxybutyrate} + \text{NAD} \xrightarrow[\text{dehydrogenase}]{\text{D-3-Hydroxybutyrate}} \text{Acetoacetate} + \text{NADH} + \text{H}^+$$

$$\text{NADH} + \text{INT (oxidized)} \xrightarrow{\text{Diaphorase}} \text{NAD} + \text{INT (reduced)}$$

Reagents

Enzyme (R1), Cat No. 2441
Contains ß-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

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 ß-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 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.

Procedure

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.

Calculation:

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

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

In order to assure consistent performance, it is recommended that at least two (2) levels of control be assayed at least once per day. Controls should also be assayed after calibration, instrument maintenance and after loading a new lot of reagent. It is suggested to use TDM/ß-hydroxybutyrate Tri-Level Controls, Cat. No. 2460-605, or TDM/ß-hydroxybutyrate Bi-Level Controls, Cat. No. 2465-605 available from Stanbio Laboratory.

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.[12]

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).[4,5] Other ranges have also been reported.[13]

Performance Characteristics

Linearity (Application dependent): The procedure described above is linear to 4.5 mM (46.8 mg/dL) ß-hydroxybutyrate. For higher concentrations, dilute the sample with distilled water or normal saline (0.9%). Repeat the assay and multiply the results by the dilution factor.

Sensitivity: Concentrations of ß-hydroxybutyrate of 0.18, 0.28, and 0.38 mM (1.8, 2.9, and 3.9 mg/dL) can be clearly distinguished at the 99.9% confidence limit.

Precision: Precision studies were conducted using two serum pools containing 0.25 mM (2.6 mg/dL) and 1.0 mM (10.4 mg/dL) ß-hydroxybutyrate. The following results are averages of eighteen determinations.

	Mean (mM)	SD (mM)	CV (%)
Within day	0.29	0.005	1.7
	1.09	0.015	1.4
Between day	0.26	0.014	5.2
	1.05	0.018	1.7

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Procedure No. 2440CE • RBR.2440.CE.04 • Revision: 03/19