Stanbio ß-Hydroxybutyrate LiquiColor® Procedure No. B2440

Intended Use

For the Quantitative Determination of ß-Hydroxybutyrate in Serum or Plasma.

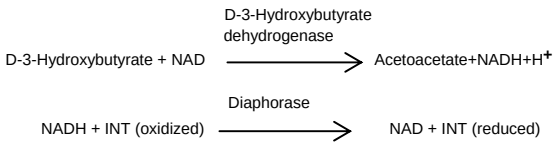
Summary and Principle

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 78% 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 and 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.



Reagents

BHB LiquiColor® Enzyme (R1), Ref No. 2441
Contains ß-hydroxybutyrate dehydrogenase and diaphorase enzymes.

BHB LiquiColor® Catalyst (R2), Ref No. 2442
Contains NAD, INT, and oxalate.

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

Precautions: *For In Vitro Diagnostic Use*
Normal precautions exercised in handling laboratory reagents should be followed. Do not pipette by mouth.

Reagents contain sodium azide which may be toxic if ingested. Sodium azide may also react with lead and copper plumbing to form highly explosive metal azides. Refer to Material Safety Data Sheet for any updated risk, hazard or safety information. Dispose of used or expired reagents according to your laboratory and governmental requirements.

Reagent Preparation: BHB Enzyme (R1) and BHB Catalyst (R2) liquid reagents are supplied ready-to-use for analyzers capable of dispensing 2 separate reagents.

Reagent Storage and Stability: Reagents are stable until the expiration date on their respective labels, when properly stored at 2-8°C. Both the Enzyme (R1) and Catalyst (R2) should be discarded if either appears cloudy or contains particulate matter.

Specimen Collection and Storage

All specimens used in this test should be considered potentially infectious. Universal precautions as they apply to your facility should be used for handling and disposal of materials during and after testing. Clear unhemolyzed serum is the specimen of choice. Plasma collected with EDTA, heparin, or sodium fluoride can be used in this assay. Whenever possible specimens should be separated and analyzed on the day of collection. 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.

Materials Provided

BHB LiquiColor® Enzyme (R1), BHB LiquiColor® Catalyst (R2) and BHB Standard.

Material Required But Not Provided

Spectrophotometer capable of absorbance reading at 505 nm and 1 cm lightpath
Constant temperature block or bath, 37°C, or temperature controlled cuvette
Accurate pipetting devices Test tubes Interval timer

Automated Procedure

Applications for automated analyzers are available by contacting Stanbio's Technical Service Department.

Manual Procedure

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

1) Incubate the needed amount of Reagent 1 (Enzyme) at 37 °C for 3 minutes.
2) To two 3 mL cuvettes, add 2.15 mL of Reagent 1 (Cuvettes 1 and 2).
3) To cuvette 1, add 60 µL of sample to be tested and immediately measure the OD at 505nm (T_0).
4) To the same cuvette 1, add 0.36 mL of Reagent 2 (Catalyst) and measure the final OD at 505nm (T_f) at 5 minutes.
5) To cuvette 2, add 60 µL of BHB Standard and immediately measure OD at 505nm (T_0, std).
6) To the same cuvette 2, add 0.36 mL of Reagent 2 and measure the final OD at 505nm (T_f, std) at 5 minutes.
7) Subtract T_0 from T_f to obtain OD (5min) for both serum and standard.

Calculation:

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

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.

Calibration: Calibration is required. If calibration is required by the instrument manufacturer, follow the calibration guidelines to calibrate your analyzer.

Quality Control: Stanbio recommends the use of commercially available controls with ß-hydroxybutyrate values assayed by this method for verifying accuracy and precision. It is suggested to use TDM/ß-hydroxybutyrate Tri-Level Controls, Ref. No. 2460-605 or TDM/ß-hydroxybutyrate Bi-Level Controls, Ref. No. 2465-605, available from Stanbio Laboratory.

Limitations

The incorporation of oxalic acid in this reagent eliminates interference of lactic dehydrogenase and lactate 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: The manual procedure described is linear to 4.5 mM (46.8 mg/dL) ß-hydroxybutyrate. For automated analyzers the linearity is application dependent. For higher concentrations, dilute the sample with distilled water. 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.

Within-Run

	Serum 1	Serum 2
Mean (mM)	0.29	1.09
Std. Deviation	0.005	0.015
CV (%)	1.7	1.4

Total Precision

	Serum 1	Serum 2
Mean (mM)	0.26	1.05
Std. Deviation	0.014	0.018
CV (%)	5.2	1.7

References

1. Foster DW and McGarry, N Eng J Med 309, 159 (1983).
2. Persson B, Scand J Clin Lab Invest 25, 9 (1969).
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6. Stephens JM, Sulway MJ and Watkins PJ, Diabetes 20 (7), 485 (1971).
7. Harano Y, Kosugi K, Hyosu T, Uno S, Ichikawa Y and Shigeta Y, Clin Chem Acta 134, 327 (1983).
8. MacGillivray MH, Li PK, Lee JT and et al., J Clin Endocrinol Metab 54, 665, (1982).
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10. Williamson DH, Mellenby J and Krebs HA, Biochem J 82, 90 (1962).
11. Zivin JA and Snarr JF, Anal Biochem 52, 456 (1973).
12. McMurray CH, Blanchflower WJ and Rice DA, Clin Chem 30/ 3, 421 (1984).
13. Hansen JL and Freier EF, Clin Chem 24/3, 475 (1978).

Index of Symbols

	Attention, see instructions for use	Σ	Tests per kit		Manufactured by
IVD	For *in vitro* diagnostic use only		Use by		Do not reuse
	Store between temperature indicated	LOT	Lot Number	REF	Reference No.

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Beckman Synchron® LX/DX BHOB Application

Test Name:	BHOB	User def. No:	#
Chem. Name:	BHOB		
Chemistry parameters			
Reaction Type:	EndP 2	Calculation Factor:	1.000
Unit:	mmol/L	No of Calibrators	2
Precision.:	x.xx	Calibrator 1:	0.0 (H_20)
Reaction Dir.:	Positive	2:	1.0
		3:	
Math. Model:	Linear	4:	
		5:	
Prim. Wavelength:	520	6:	
Sec. Wavelength:	700		
		Cal. Time Limit:	336 hr

First inject [B] Vol.:	200 µL		
Second inject [ ] Vol.:	None	Inject Time	-180 sec
Third inject [C] Vol.:	40 µL	Inject Time:	240 sec
Sample Volume:	3 µL		

Reagent Blank	Initial	Reaction 1	Reaction 2
Start Read:	Start Read:	Start Read:	
200 sec.	540 sec.	540 sec.	
End Read:	End Read:	End Read:	
220 sec.	560 sec	560 sec.	

Usable result range		ORDAC
Lower limit:	0.05	0.0
Upper limit:	8.00	99999.9

Error detection limits	Blank	Reaction 1	Reaction 2
ABS Low Limit	-1.500	-1.500	-1.500
ABS High Limit	2.200	2.200	2.200
Rate Low Limit	-1.500	-1.500	-1.500
Rate High Limit	2.200	2.200	2.200
Mean Deviation	2.200	2.200	2.200

Substrate Depletion:			
Initial Rate:	99.99	Delta Abs.:	2.200

User defined

The stability of the reagent on board the analyser is 30 days provided contamination and evaporation are avoided.