Stanbio Laboratory

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.

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

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

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
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 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.

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/B-Hydroxybutyrate Tri-Level Controls (Catalog No. 2460-605) or TDM/B-Hydroxybutyrate Bi-Level Controls (Catalog No. 2465-605). See the instructions for use for use and control ranges.
2) Three or two 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.
a) Repeat the same control but do not report patient results unless the repeated control results are within the established limits.
b) If the repeated control results are still outside of established limits, use a fresh bottle of control and repeat the test. If the results are within range, report patient results.
c) If the results from the fresh controls are still out of range, recalibrate the analyzer and repeat the controls. If the controls are within range, repeat test with patient specimens.
d) If after recalibrating on the existing reagent the control results are still out of range, recalibrate on fresh reagent and repeat the controls. If the control results are in range, repeat test with patient specimens.
e) If none of the preceding produces acceptable QC results, contact Stanbio Laboratory Technical Service Department (800-531-5535).
4) The mean value and expected range found on the instructions for use are derived from interlaboratory data. The expected range includes instrument, reagent, and laboratory variations. This laboratory's mean of several determinations may not duplicate the mean value found in the instructions for use, but should fall within the expected range.
5) Each laboratory should establish its own mean and precision parameters.

Results

Values are derived by the following equation:

$$\frac{\text{Au}}{\text{As}} \times 1\ \text{mmol/L} = \beta\text{hydroxybutyrate (mmol/L)}$$

Where Au and As are the absorbance values of the unknown and standard respectively, 1 is the concentration of the standard.

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-hydroxy-butyrate 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]

Measuring Range

The measuring range is 0.02 to 6.0 mmol/L.

Performance Characteristics for Ortho VITROS 4600

Sensitivity Conducted in accordance with CLSI EP17-A2.	
Limit of Blank	≤ 0.02 mmol/L
Limit of Detection	0.016 mmol/L
Limit of Quantitation	0.031 mmol/L at 10% CV

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.		
Within run	Control Level 1	Control Level 2
Mean (mmol/L)	0.194	4.209
Coefficient of variation (%)	2.58	0.67
Total run	Control Level 1	Control Level 2
Mean (mmol/L)	0.194	4.209
Coefficient of variation (%)	3.09	0.86

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 = 0.9542x – 0.0123 with correlation coefficient of 0.9947

Performance Characteristics for Ortho VITROS 5600

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 - 6.0 mmol/L

Precision Conducted in accordance with CLSI EP05-A3 over 22 days.		
Within run	Control Level 1	Control Level 2
Mean (mmol/L)	0.188	4.232
Coefficient of variation (%)	1.06	0.57
Total run	Control Level 1	Control Level 2
Mean (mmol/L)	0.188	4.232
Coefficient of variation (%)	1.06	0.90

Method comparison 105 samples (85 serum/20 plasma) were run on Ortho VITROS 5600 compared to Sirrus analyzer, in accordance with CLSI EP09-A3.
y = 0.97x – 0.0227 with correlation coefficient of 0.9972

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

1. Foster DW and McGarry, N Eng J Med 309, 159 (1983).
2. Persson B, Scand J Clin Lab Invest 25, 9 (1969).
3. Wildenhoff KE, Clin Chem 24, 475 (1978).
4. Koch DD and Feldbruegge DH, Clin Chem 33 (10), 1761 (1987).
5. Li PK, Lee JT, MacGillivray MH, Schaefer PA and Siegel JH, Clin Chem 26(12), 1713 (1980).
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).
9. Alberti KGMM and Hockaday TDR, Brit Med J 2, 565 (1972).
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).

For Technical Service call: 800-531-5535 • (830) 249-0772
Fax (830) 249-0851 • e-mail: stanbiolab@ekfdiagnostics.com
http://www.ekfusa.com
Stanbio Laboratory • 1261 North Main Street • Boerne, Texas 78006

IFU-00055.0 • Procedure No. 2440