POINTE SCIENTIFIC, INC.
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β-Hydroxybutyrate Reagent Set

Intended Use
For the quantitative determination of β-hydroxybutyrate in serum or plasma. For *in vitro* diagnostic use only.

Summary and Explanation of Test
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 β-hydroxybutyrate.

Ordinarily, β-hydroxybutyrate is the ketoacid present in the greatest amount in serum. It accounts for approximately 75% of the ketone bodies that also contain acetoacetate and acetone.[2,3,4] 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.[5,6,7,8]

In diabetics, the measurement of β-hydroxybutyrate as well as 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.[9] A specific enzymatic assay for β-hydroxybutyrate is therefore extremely important in the assessment of ketosis.

Principle
Enzymatic quantitation of β-hydroxybutyrate by β-hydroxybutyrate dehydrogenase has been reported.[10,11,12] In the Pointe Scientific method, β-hydroxybutyrate (D-3-hydroxybutyrate) in the presence of NAD gets converted to acetoacetate and NADH at a pH 8.5 by β-hydroxybutyrate dehydrogenase (D-3-hydroxybutyrate dehydrogenase). At this pH, the reaction is favored to the right.[12] The NADH produced is converted to color using INT and diaphorase.

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

$$\text{NADH} + \text{INT (oxidized)} \xrightarrow[\text{Diaphorase}]{} \text{NAD} + \text{INT (reduced) color cpd. 505nm}$$

Reagents
The Pointe Scientific β-hydroxybutyrate reagent kit contains the following:
1. 1 x 50ml R1 containing β-hydroxybutyrate dehydrogenase and diaphorase enzymes.
2. 1 x 8ml R2 containing NAD, INT and oxalate.
3. 1 x 3ml standard containing 1mM sodium D-3-hydroxybutyrate

Reagent Storage and Stability
All of the above reagents are stable stored at 2-8°C until the expiration date stated on the labels. Do not use the reagents past their expiration date. Alterations in the physical appearance of the reagents or values of control materials outside of the manufacturer's acceptable range may be an indication of reagent instability.

Reagent Preparation
R1 and R2 reagents are supplied as ready to use liquids. To prepare a single liquid working reagent mix 10 parts R1 with 1.5 parts R2 (ex. 10.0ml R1 with 1.5 ml R2).

Warnings and Precautions
For *in vitro* diagnostic use only. Avoid skin contact with reagents. If this occurs, wash immediately with water.

Specimen Collection and Preparation
Serum or plasma collected with EDTA, heparin or sodium fluoride can be used in the assay. Serum or plasma β-hydroxybutyrate levels are stable at least one week if kept refrigerated. (2-8°C)

Interferences
Lactate dehydrogenase and lactate have been shown to interfere with the assay. The incorporation of oxalic acid is this reagent eliminates this interference as reported.[12] No significant changes in values were observed when the following analytes were added to serum containing 0.5mM β-hydroxybutyrate.

Analyte	% Recovery
Glucose (2000 mg/dl)	96
Acetoacetic acid (5 mM)	96
Creatinine (5 mg/dl)	106
Ascorbate (3mg/dl)	106
Bilirubin (10 mg/dl)	96
Uric Acid (16 mg/dl)	102
Triglyceride (417 mg/dl)	104
Cholesterol (314 mg/dl)	94
Lactate Dehydrogenase (1515 U/ml)	93
Sodium Lactate (96 mg/dl)	99

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

Materials Provided
Refer to "Reagents"

Materials Required but not Provided
1. 10 ml Micropipettor
2. 1 ml Pipette or Dispenser
3. Glass or plastic Test Tubes to hold 1.0 ml
4. β-hydroxybutyrate controls
5. Temperature controlled incubator
6. Spectrophotometer capable of reading at 505nm.

Procedure (Automated)
Refer to specific instrument application instructions.

Procedure (Manual)
The Pointe Scientific enzymatic β-hydroxybutyrate reagent provided can be used in a five-minute procedure utilizing a spectrophotometer that reads at 505 nm.

Conditions:		
	Wavelength	505nm
	Temperature	37°C
	Mode	Endpoint
	Reagent Volume	1.0 ml
	Sample volume	25 ul
	Incubation	5 min.

1. Prepare working reagent according to "Reagent Preparation" instructions.
2. Label tubes, "Standard", "Control", "Patient", etc.
3. Pipette 1.0 ml of working reagent into required number of labeled test tubes. Incubate at 37°C for 5 minutes.
4. Add 25 ul Standard, controls and patients to the appropriate tests and incubate at 37°C for 5 minutes.
5. Zero spectrophotometer with a reagent blank.
6. Read and record absorbances of all tubes at 505 nm.

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.

Calibration

Use the β-hydroxybutyrate standard provided.

Calculations

$$\beta\text{-hydroxybutyrate (mM)} = \frac{\text{OD (5min) serum}}{\text{OD (5min) standard}} \times \text{Standard concentration}$$

To convert results to mg/dL divide the value obtained in mM by 0.096

Limitations

1. See "Interferences".
2. The procedure described above is linear to 4.5mM (46.8 mg/dl) β-hydroxybutyrate. For higher concentrations dilute the sample with deionized water, repeat the assay and multiply the results by the dilution factor.

Quality Control

The reliability of test results should be monitored whenever patient samples are assayed using a standard and quality control materials analyzed in the same manner employed for the unknowns. We suggest the use of commercially available β-hydroxybutyrate controls with an assayed range. If controls do not fall into the assayed range, patient values from that run should not be reported. The run should be repeated, making sure that all mixing and handling instructions are strictly followed.

Linearity of the assay should be verified with a commercial linearity check set, or dilutions of a high specimen, at least every six months.

Expected Values

The quantitation of β-hydroxybutyrate is important in cases of ketoacidosis. In studies of healthy individuals who had fasted for 12 hours before blood collection, the range of β-hydroxybutyrate was found to be from 0.02 mM (0.2mg/dl) to 0.27mM (2.81mg/dl).[4,5] Other ranges have also been reported.[13]

Performance

1. Linearity: The β-hydroxybutyrate assay is linear to 4.5 mM (46.8 mg/dl).
2. Comparison: A study of 57 samples using Pointe Scientific and the Sigma method gave the following correlation. (y=Pointe Scientific, x=Sigma): y = 1.05x + 0.25mM, N = 57, r = 0.9922, Sxy = 0.1055mM.
3. Precision: Studies were conducted using two serum pools containing 0.25mM (2.6 mg/dl) and 1.0mM (10.4 mg/dl) β-hydroxybutyrate. The following results are averages of eighteen determinations:

Within day:

Level	Mean (mM)	Std. Dev. (mM)	% C.V.
1	0.29	0.005	1.7
2	1.09	0.015	1.4

Day to day:

Level	Mean	Std. Dev.	% C.V.
1	0.26	0.014	5.2
2	1.05	0.018	1.7

4. Sensitivity: Concentrations of β-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% confidence limit.

References

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Pointe Scientific maintains that this product conforms to the information contained in the insert. The purchaser must determine the suitability of the product for their particular use. Use only in accordance with labeling instructions.

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