Intended Use
For ***In Vitro*** **Diagnostic** use in the automated, quantitative determination of β-Hydroxybutyrate in serum or plasma.

Clinical Significance (1-5)
Measurement of β-Hydroxybutyrate (β-HBA) is of considerable clinical value in the diagnosis and management of ketosis. During ketosis β-Hydroxybutyrate increases to a greater extent than the other two ketoacids, acetoacetate and acetone. Reports have shown that β-HBA is a better indicator than acetoacetate and acetone for monitoring treatment of diabetic ketoacidosis or even for detecting sub clinical ketosis. Also, accurate laboratory measurement of acetoacetate and acetone is difficult because of their inherent instability, whereas β-HBA is comparatively stable.

Method Principle (1, 5, 6-9)
Several procedures have been reported in the literature for measuring β-HBA in serum. Catachem's procedure is based on the enzymatic procedure described by David D. Koch and Donald H. Feldbruegge. In this procedure β-Hydroxybutyrate dehydrogenase (HBDH: EC 1.1.1.30) catalyzes the reaction where β-HBA is converted to acetoacetate with concomitant reduction of NAD to NADH. The increase in absorbance is monitored at 340nm. The delta absorbance produced is directly proportional to the concentration of β-HBA in the serum sample. The reaction scheme below illustrates the reactions that take place in this β-HBA procedure.

$$\text{D-β-HBA} + \text{NAD} \xrightarrow{\text{β-HBDH}} \text{Acetoacetate} + \text{NADH}$$

REAGENTS
B-HBA Sample Diluent Reagent (R1)
Each liter contains:

Buffer	
Magnesium Chloride	4.0 mmol
Oxalic Acid	10.0 mmol
β -HBDH	≥5 KU
Stabilizer and nonreactive ingredients	

B-HBDH Enzyme Reagent (R2)
Each liter contains:

Buffer	
NAD	5 mmol
Stabilizer and nonreactive ingredients	

Precautions
Handle these reagents using good laboratory practice. **DO NOT PIPETTE REAGENT BY MOUTH.** Avoid contact with skin and eyes. If contact occurs, wash affected area with plenty of cold water. Clean spills immediately.

Reagent Storage and Stability
Store the β-HBA Sample Diluent (R1) and the β-HBDH Enzyme Reagent (R2) at 2-8°C. When stored as directed, these reagents are stable until expiration date stated on the label.

Working Reagent Preparation
Catachem β-HBA reagents are packaged ready for use. No preparation is required. Upon opening containers, the Working Reagents are stable at 2-8˚C for 60 days.

Reagent Indications of Deterioration
- Reagent absorbance > 0.6 O.D
- Quality control values out of assigned ranges

If these reagent characteristics are observed call your technical representative.

Specimen Collection and Stability
Clear unhemolyzed sera are the specimens of choice. Serum should be separated immediately from the clot and analyzed promptly or stored at 2-8°C. β-HBA serum is stable 7 days at room temperature at 18-26ºC and 14 days refrigerated at 2-8°C and for several months frozen at -20°C. (7, 10).

Procedure
These instructions are outlined for performing the β-HBA assay using a manual procedure.

Materials Required But Not Provided
- Manual analyzer
- Catachem Calibrator and Quality Control materials with assigned β-HBA values

Quality Control
To monitor the quality performance of the procedure used, Catachem B-HBA Control Level I and Control Level II with assigned β-HBA values should be included in the assay procedure each time the procedure is run.

Interfering Substances (1, 11, 12)
The Catachem β-HBA method is not significantly affected by bilirubin, hemolysis, glucose, or acetoacetic acid. Grossly hemolyzed samples (as high as 11,000 mg/dl) depressed β-hydroxybutyrate levels by as much as 8.3 mg/dl. Lipemia with triglycerides levels greater than 1000 mg/dl interferes with determination of β-hydroxybutyrate. Triglyceride levels less than 1000 mg/dl has negligible effect. A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S.Young et al.

Procedure Limitations
Samples with β-HBA values greater than 75 mg/dL should be diluted 1:2 with physiological saline and reassayed. Multiply results obtained by 2 to adjust for the sample dilution.

Assay Procedure

1. Label cuvettes or appropriate test tubes as: a) Calibrator blank (CAL-BLK), b) Calibrator (CAL), c) Control 1 blank (C-1BLK), d) Control 1 (C1), e) Control 2 blank (C-2BLK), f) Control 2 (C2), g) Sample blank (SAMP BLK), h) Sample (SAMP).
2. Pipette the reagent and sample volumes into the cuvettes or test tubes as shown in table below. Pipette β-Hydroxybutyrate Sample Diluent (R-1) first, followed by β-Hydroxybutyrate Enzyme Reagent (R-2) and the sample or water last.
3. After all reaction components are in the cuvettes, quickly mix all cuvettes without delay.
4. Set a timer for exactly 2.0 minutes.
5. At the end of the 2.0 minutes, read all cuvettes at 340nm. Record all absorbencies.

	CAL BLK	CAL	C-1 BLK	C-1	C-2 BLK	C-2	SAMP BLK	SAMP
	ml	ml	ml	ml	ml	ml	ml	ml
RGT 1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
RGT 2	0.0	0.10	0.00	0.10	0.00	0.10	0.00	0.10
SAMP	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
H2O	0.10	0.0	0.10	0.0	0.10	0.0	0.10	0. 0

Calculations and Results

$$\beta\text{-Hydroxybutyrate mg/dL} = \frac{\Delta\text{- Abs. Samp.}}{\Delta\text{- Abs. Cal.}} \times \text{Cal. mg/dL}$$

Example:

	Samp. Abs.	Blk. Abs.	Δ-Abs.
Sample	0.400	0.300	0.100
Calibrator	0.150	0.030	0.120

Calibrator assigned value = 15 mg/dL

$$\text{Sample B- β-Hydroxybutyrate mg/dL} = \frac{0.100}{0.120} \times 15 \text{ mg/dL}$$
$$= 12.5 \text{ mg/dL}$$

Expected Values:

Minimum: 1.9 mg/dL (0.02 mmol/L)
Maximum: 26 mg/dL (0.27 mmol/L)

The values given here are only to be used as a guideline. It is recommended that each laboratory establish the normal range for the species in question and the area in which the laboratory is located (1)

Method Performance Characteristics

Sensitivity: Using a path length of 1 cm, a □absorbance of 0.003± 20% per mg/ml should be obtained.

Linearity: In this procedure there is not significant nonlinearity over the range of 0-75mg/dL.

Accuracy

Correlation studies were carried out between this automated β-Hydroxybutyrate method (Y) and a reference automated β-Hydroxybutyrate procedure based on the β-Hydroxybutyrate dehydrogenase reaction (X). Canine serum samples were assayed and the results compared by the least square regression. The following statistics were observed:

N	=	14
Range	=	4.5-25
Mean X	=	10.63
Mean Y	=	12.14
Y	=	0.957x - 0.27
r	=	0.993
Sy.x	=	0.43

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