

Intended Use

Mission's Beta-Hydroxybutyrate Reagent is for the quantitative measurement of beta-hydroxybutyrate (BHB) in urine.

For Research Use Only. Site validation required for Lab Developed Test (LDT), 510K approval pending.

Summary and Principle

Out of the three ketone bodies - BHB, acetoacetate and acetone - which are formed in the liver during increased fat breakdown, BHB is the most abundant ketone (~75–80% of total ketones) in ketoacidosis. BHB is a key marker in detecting the severity of diabetic ketoacidosis. Reports have shown that BHB 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 BHB is comparatively stable.¹⁻⁴

The following procedure is based on the enzymatic procedure described by Williamson et al.⁵ In this procedure beta-hydroxybutyrate dehydrogenase catalyzes the reaction where BHB is converted to acetoacetate with concomitant reduction of NAD to NADH. The NADH that is produced is further converted to a colored compound using the enzyme diaphorase and the dye, INT (2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium chloride). The increase in absorbance is monitored at 505 nm. The absorbance produced is directly proportional to the concentration of BHB in the urine sample.

Reagents

Reagents are packaged ready for use. No preparation is required.

Reagent 1 contains: buffer, beta-hydroxybutyrate dehydrogenase and diaphorase enzymes.

Reagent 2 contains: buffer, INT, NAD and oxalate.

Reagents Stability and Storage

Store Reagent 1 and Reagent 2 at 2-8°C. When stored as directed, these reagents are stable until expiration date stated on the label.

Cautions

Handle these reagents using good laboratory practice. Avoid contact with skin and eyes. If contact occurs, wash affected area with plenty of cold water. Clean spills immediately.

Instrumentation

The Mission BHB Reagent Kit is for use on Mission CA200¹ and Beckman AU680 analyzers. Refer to instrument procedure instructions in the instrument manual provided with the specific analyzer.

Specimen Collection and Handling

Use a clean, dry, sterile plastic container with a tightly sealed lid. Test within 2 hours of collection.

Store samples protected from light and heat as ketones, including BHB, degrade with exposure. Refrigeration is essential if there is any delay in testing.

Quality Control

Store and handle reagents properly before and during use. Every laboratory should establish its own test requirements using controls and calibrators. Mission Diagnostics provides calibrators to meet your program needs and which conform to NLCP Guidelines⁶:

MD-101208 – Beta-hydroxybutyrate Calibrator

Specificity, Limitations, and Interferences

Improper storage leads to degradation of BHB. Bacteria can consume ketones, lowering results. Light exposure degrades ketone bodies. pH extremes (very acidic or alkaline urine) may alter enzymatic activity.

Typical Performance Characteristics

The following performance data was obtained using the Mission CA200 and Beckman AU680 analyzers. Other instruments may yield different performance data.

Linearity

The following results were obtained on Mission CA200 and Beckman AU680 analyzers using the Mission BHB Reagent Kit on samples containing 1.80, 3.65, 7.25, 14.5, 33, 50 and 65 mg/dL BHB. The table below includes mean, standard deviation (SD) and coefficient of variation (CV) for each value.

Mean (mg/dL)	SD	CV%
1.95	0.01	0.6
3.88	0.02	0.6
7.64	0.02	0.2
14.61	0.05	0.4
31.77	0.30	0.9
49.00	0.25	0.5
65.92	0.80	1.2

¹ Also known as Zybion EXC200 Analyzer

Precision

Studies were performed on Mission CA200 and Beckman AU680 analyzers. The precision of the assay was evaluated following a modification of NCCLS protocol EPT-T2. The within-run precision data was obtained by running two samples in replicates of 20 on the same day. The run-to-run data was obtained by running two samples in replicates over a five-day period.

Within-Run			Run-to-Run		
Mean (mg/dL)	SD	CV%	Mean (mg/dL)	SD	CV%
8.09	0.03	0.4	8.17	0.19	2.3
24.50	0.13	0.5	25.13	0.60	2.4

Analytical Specificity

Cross contamination studies have not been performed. Certain reagent/instrument combinations used in sequence with this assay may interfere with reagent performance and test results. The existence of, or effects of, any potential cross contamination issues are unknown.

Test Conditions

For the data presented in this insert, studies using this reagent were performed on Mission CA200 and Beckman AU680 analyzers using the parameters listed below.

Limitations

A sample with a BHB concentration exceeding the analytical measuring range should be diluted with deionized water and reanalyzed incorporating the dilution factor in the calculation of the value.

The limit of quantification (LOQ) must be determined by each laboratory according to their SOP.

Calibration

Calibration material should be used to calibrate the procedure. The frequency of calibration using an automated system is dependent on the system and the parameters used. If control results are found to be out of range, the test may need to be re-calibrated.

Method Parameters

Analyzer Specific Settings

Method type:	Endpoint
Slope:	positive
Units	mg/dL
DOM wavelength	505
SUB wavelength	660
Sample volume	3
R1 volume	150
R2 volume	24
Blank rxn read (cycles)	16 - 20
Sample rxn read (cycles)	36 - 40

Calibration Settings

Calibration Type	Linear
Reagent Blank required	No
Calibrator 1 (water)	0
Calibrator 2	15

Analytical Measuring Range (AMR)

Range: (low)	LOQ
Range: (high)	65

BIBLIOGRAPHY

1. Rifai, N., Horvath, A. R., & Wittwer, C. T. (Eds.). (2017). *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics* (6th ed.). St. Louis, MO: Elsevier.
2. McPherson, R. A., & Pincus, M. R. (Eds.). (2022). *Henry's Clinical Diagnosis and Management by Laboratory Methods* (24th ed.). Philadelphia, PA: Elsevier.
3. Mayo Clinic Laboratories. (n.d.). *Beta-Hydroxybutyrate, Quantitative, Serum or Plasma*.
4. American Diabetes Association. (2024). *Standards of Care in Diabetes—2024. Diabetes Care*, 47 (Suppl. 1), S1–S300.
5. Williamson D.H, Mellenby J and Krebs H.A., *Biochem. J.* 82, 90 (1962).
6. National Laboratory Certification Program. General Laboratory Inspection Checklist / Report. OMB No. 0930-0158. November 2002.