

## Beta-hydroxybutyrate: New Test for Ketoacidosis

Salvador F. Sena, Ph.D., DABCC  
Medical Director, Clinical Chemistry

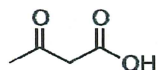
The Clinical Chemistry area of the laboratory is now performing a new test for beta-hydroxybutyrate (BHB) in serum. The BHB test will replace the classic nitroprusside test for serum ketones. BHB is measured by an enzymatic spectrophotometric assay on the Vitros 5,1 FS chemistry analyzer. In contrast to the qualitative results reported for serum ketones, BHB results are reported quantitatively in mmol/L (reference interval  $\leq 0.3$  mmol/L). BHB is available 24/7 with a stat turnaround time of 45 minutes. Both BHB and ketones will be performed with all orders for serum ketones for an interim period to allow clinicians to become accustomed to BHB; following this period, the ketones test will be discontinued.

### Biochemistry of Ketogenesis and Ketone Bodies

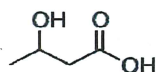
In states of carbohydrate deprivation (starvation, low carbohydrate diet, digestive disorders, prolonged vomiting), decreased utilization (diabetes mellitus), glycogen storage diseases and alkalosis, the body turns to fat metabolism to meet its energy requirements. Fat or long-chain fatty acids from adipose tissue are metabolized by hepatocytes via beta-oxidation to sequentially remove two-carbon units (acetyl-CoA) from the fatty acid chain. Normally, acetyl-CoA is further oxidized in the citric acid cycle but when the capacity of this cycle is exceeded, ketogenesis occurs to produce three so-called "ketone bodies": acetone, acetoacetate (AcAc) and beta-hydroxybutyrate (BHB), also known as 3-hydroxybutyrate (see figure). Only acetone and AcAc are true ketones that are detected by the widely used nitroprusside colorimetric spot test for ketones; BHB is not a ketone and is not detected by this test.



Acetone (2%)



Acetoacetic acid (20%)



Beta-hydroxybutyrate (78%)

### KEY POINTS

- ✚ BHB is the major "ketone body" produced in ketosis; AcAc and acetone are produced in smaller amounts.
- ✚ The nitroprusside test for ketones detects AcAc and acetone but does NOT detect BHB.
- ✚ BHB levels correlate more closely than serum ketones with the degree of anion gap elevation in ketoacidotic states and with resolution of ketoacidosis.
- ✚ AcAc may be initially low in untreated ketotic patients but may paradoxically increase in response to therapy.
- ✚ Normal BHB levels are  $\leq 0.3$  mmol/L and are typically  $> 2.0$  mmol/L in patients with ketoacidosis.
- ✚ BHB levels can also be used in monitoring ketogenic diets, monitoring hypoglycemia and in diagnosing insulinoma.

In healthy individuals, ketone bodies are present in very low concentrations (total serum ketones <0.5 mmol/L) with approximately equimolar amounts of the principal ketone bodies, BHB and AcAc. Acetone is formed from decarboxylation of AcAc and usually accounts for only 2% of the total ketone bodies. Acetone cannot be metabolized to acetyl-CoA; it is excreted in the urine or exhaled and is responsible for the “fruity” breath odor of ketotic patients. AcAc normally accounts for about 20% of the circulating ketone bodies in ketotic patients and is spontaneously converted to acetone and CO<sub>2</sub>. AcAc is also metabolized by the enzyme beta-hydroxybutyrate dehydrogenase into BHB. BHB accounts for the majority (about 78%) of the total ketone body pool.

### **Clinical Utility**

Excess formation of BHB results in a state of ketoacidosis and BHB is the primary compound that is responsible for the elevated anion gap found in ketotic patients. The equilibrium between AcAc and BHB is shifted towards formation of BHB in any condition that alters the redox state of hepatic mitochondria to increase concentrations of NADH such as hypoxia, fasting, metabolic disorders (including DKA) and alcoholic ketoacidosis. Thus, AcAc concentrations can be low in untreated ketotic patients and may actually increase in response to therapy. Because nitroprusside tests detect only AcAc and acetone and do not detect BHB, these tests may provide misleading clinical information by underestimating the total serum ketone body concentration in patients presenting with anion gap metabolic acidosis.

Since BHB is the predominant ketone body, it is the most sensitive marker for detection of ketoacidosis. The American Diabetes Association has recommended BHB analysis as the preferred method for diagnosing and monitoring DKA. The upper reference limit for BHB is assay dependent and ranges from 0.3-0.5 mmol/L. Patients with well-documented DKA (serum HCO<sub>3</sub><sup>-</sup> <17 mmol/L, arterial pH <7.3, serum glucose >250 mg/dL) typically have BHB concentrations >2 mmol/L. Starvation ketosis and alcoholic ketoacidosis have normal-moderate glucose increases and HCO<sub>3</sub><sup>-</sup> is usually not less than 18 mmol/L. BHB levels have also been shown to be useful for assessing patients that may need additional insulin therapy following intravenous insulin drips for DKA and can be used to establish endpoints for IV therapy. Children with persistent elevations of serum BHB with negative urine ketones were shown to have a higher incidence of recurrence of ketonuria and may benefit from continuation of IV therapy.

In addition to diagnosing and monitoring therapy for ketoacidosis, clinical uses of BHB measurements also include monitoring the efficacy of a ketogenic diet, monitoring hypoglycemia, diagnosis of insulinoma and forensic applications.

**Questions?** Contact Dr. Sena at 203-739-7622 or [salvador.sena@danhosp.org](mailto:salvador.sena@danhosp.org).

### **Selected References**

1. Participant Summary, CAP Ketones Survey 2008 KET-B, College of American Pathologists, 2008.
2. Sacks DB, et al. Guidelines and Recommendations for Laboratory Analysis and in the Diagnosis and Management of Diabetes Mellitus. Clin Chem 2002;48:436-472.
3. Sheikh-Ali M, et al. Can serum beta-hydroxybutyrate be used to diagnose diabetic ketoacidosis? Diabetes Care. 2008;31:643-7.
4. Laffel L. Ketone bodies: a review of physiology, pathophysiology, and application of monitoring to diabetes. Diabetes Metab Res Rev. 1999;15:412-26.