Beta Hydroxybutyrate -A Better Test for Ketosis

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When the body does not have sufficient carbohydrates available to meet energy demands, it begins to utilize stored fat for energy production. The breakdown of fatty acids in the liver produces three compounds: ß-hydroxybutyrate (BOHB), acetoacetate, and acetone, known collectively as ketone bodies. BOHB comprises approximately 78%, and acetoacetate is approximately 20% of the ketone bodies. Acetoacetate is unstable and breaks down into carbon dioxide and acetone, which comprises the remaining 2%[1]. The "fruity" aroma of acetone is frequently noted on the breath and in the urine in individuals with ketosis.

Ketone bodies are acidic, but under normal circumstance the renal and respiratory compensatory mechanisms maintain acid-base homeostasis. In ketoacidosis, the body produces more ketones than it can compensate for. The detection of ketosis is important in several clinical conditions, the most important being the detection and monitoring treatment of patients with diabetic ketoacidosis (DKA). Other conditions include starvation or malnutrition, alcoholism, certain inborn errors of metabolism and the investigation of an unexplained increase in the anion gap.

Since 1949, ketosis has been diagnosed and monitored utilizing the nitroprusside based tests Ketosix (urine) and/or Acetest (blood).[2] These are semi-quantitative tests where urine or blood is applied to a dipstick or tablet, and after a specified time, any color change that develops is visually graded by comparison to a color chart. The results are reported as Negative, Small, Moderate or Large. The method is not very sensitive, and reading the intensity of the color change is subjective. However, the main drawback of nitroprusside based tests is that they **only** detect acetoacetate and to a lesser extent acetone. They **do not** detect the primary ketone body BOHB. In severe diabetics, the ratio of BOHB to acetoacetate may increase to approximately 8:1 Thus the nitroprusside test is insensitive for detecting the early stages of ketoacidosis.

There are a number of other shortcomings related to the nitroprusside tests. In cases of DKA with severe complications such as lactic acidosis, the equilibrium of BOHB-acetoacetate is strongly shifted towards BOHB away from acetoacetate. In such instances, the nitroprusside test may be negative or only weakly positive even though ketoacidosis is severe. As ketosis improves BOHB converts to acetoacetate causing a false indication by the nitroprusside tests which show ketosis to be increasing. Several reports conclude that the use of the Acetest may be misleading and should be avoided because the fall of acetoacetate lags behind the resolution of ketoacidosis. A study by Umpierrez et al. points out that all patients with BOHB levels of 1.1 mMol/L or less had resolved their ketosis. In contrast, 8 of 15 patients in whom ketosis had been resolved by acid-base parameters and BOHB levels still had positive serum Acetest results.[3] Studies at Henry Ford Hospital demonstrated that at BOHB levels of 1.0-1.5mMol/L with resolution of ketoacidosis the Acetest procedure still gave positive results when diluted 1:8 and even 1:16 in several cases. [4] In addition, the nitroprusside method has demonstrated susceptibility to false positive results from drug interference and false negative results due to reagent deterioration.[5] Blood testing for ketones is superior to urine testing because fluid intake and urine concentration can significantly affect urine test results, making urine testing unreliable.

The table below displays three examples of test results seen during the test evaluation and set up of the BOHB test at Bronson Methodist Hospital Laboratory where the author is employed.

	Acetest	BOHB	Venous pH	Glucose
Reference Range	**Negative**	**0.02-0.27 mMol/L**	**7.32-7.43**	**70-99 mg/dl**
Patient A	Small	11.65 H	6.99 L	530 H
Patient B	Negative	9.10 H	not tested	834 H
Patient C	Negative	0.53 H	7.39	323 H

Comparison of test results in DKA: Acetest vs BOHB

Patient Stage	Ketone body level	Acetest (old method)	BOHB (new method)
Initial presentation	High-Increasing	Negative or Weak Positive	High-Increasing
During treatment	Decreasing	High-Increasing	Decreasing
Ketoacidosis resolved	Decreasing-Normal	Positive	Decreasing-Normal

Fortunately an automated quantitative method is now available to precisely measure BOHB levels. A liquid reagent from Stanbio Laboratories [6] is available with applications for many spectrophotometric analyzers, including Beckman, Ortho, Roche and Siemens. The company also makes a small point of care analyzer for this test. In the author's laboratory the test is run on a Roche p800 Modular. With the implementation of this test, serum ketone testing by the Acetest method has been discontinued. BOHB is performed on any orders for ketone testing. The new test is run on the same sample, on the same laboratory analyzer and with the same turn-around times as for electrolytes and other general chemistry testing. The normal range for the new test is 0.02 to 0.27 mMol/L.

Quantitative, objective BOHB testing provides a better tool for differentiating metabolic acidosis and monitoring therapy. Improved clinical outcomes and enhanced cost efficiency have also been reported due to blood testing of BOHB with earlier detection of clinically significant ketosis, improved turn-around times and meaningful values for monitoring the results of therapy.

References:

1 Tietz Text Book of Clinical Chemistry, 2nd Ed. Burtis CA, Ashwod E, Eds W.B. Saunders Co., Philadelphia. 1994:971-974 & 1013.

2 Acetest® and Ketostix® are registered trademarks of Bayer Corporation, Elkhart, Indiana.

3 Umpierrez GE, Watts NB, Phillips LS. Clinical utility of ß- hydroxybutyrate determined by reflectance meter in the management of diabetic ketoacidosis. Diabetes Care. 1995; 18(1): 137-138.

4 Foreback CC, Ph.D, Director Clinical Chemistry/ Pathology, Henry Ford Hospital, Detroit, MI. White Paper, Clinical Effectiveness of ß-hydroxybutyrate Assays in a Clinical Decision Unit, 1998

5 Csako, et. al. Unrecognized false-positive ketones from drugs containing free-sulfydryl groups(s). JAMA, 1993;269(13):1364.

6 Stanbio Laboratories, Boerne Texas www.stanbio.com