

Beta Hydroxybutyrate: A Better Test for Ketosis

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: β -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 Ketostix (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 1.0-1.5 mMol/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.

Table 1 below displays three examples of test results seen during the test evaluation and set up of the BOHB test at Bronson Methodist Hospital Laboratory and Table 2 contrasts the Acetest and BOHB test based on the patient stage.

Table 1. Select patient results illustrating discrepancies between the Acetest and BOHB levels.

	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

Table 2. Comparison of tests results in DKA with Acetest vs. BOHB method based on patient stage

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. With the implementation of this test, serum ketone testing by the Acetest method has been discontinued. BOHB will be 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 0.02-0.27 mMol/L.

Quantitative, objective BOHB testing provides a better tool for differentiating (continued on back)

Fat and Sweet — Sodium Measurements in Extreme Diabetic Conditions

Two conditions frequently seen in diabetics, hyperlipidemia and hyperglycemia, may have profound effects upon the measurement of serum sodium (Na⁺) levels. In cases of hyperlipidemia where the triglyceride concentration is often greater than 1000 mg/dl, the Na⁺ result may be falsely lowered depending upon the test method used in the laboratory. Most analyzers testing serum or plasma use indirect ion specific electrode (ISE) methods. Indirect ISE methods are susceptible to the solvent exclusion effect. Electrolytes are almost entirely associated with plasma water (the solvent) and are excluded from solids (proteins and lipids). In an indirect ISE test, the analyzer pipettes a precise volume of patient sample into a diluent. The concentration of Na⁺ is measured and the result is calculated taking the dilution factor into account and assuming no interference from solids. However, the presence of excessive solids in the sample (as in the case of extreme lipemia) “takes up space” that would otherwise be occupied by Na⁺ laden water. This leads to falsely low Na⁺ results (pseudohyponatremia).

To obtain accurate sodium results on severely lipemic samples, there are two options: ultracentrifugation and direct ISE testing. In ultracentrifugation, the sample is processed in a special centrifuge at 100,000 x g to separate the lipids and re-test the remaining plasma.¹ Ultracentrifuges are not available in most laboratories. The second option is to test the lipemic sample by a direct ISE method. Direct ISE methods are often available on whole blood analyzers such as those used in blood gas analysis. In these systems, the sample is tested without the analyzer making a dilution and the activity of the sodium is measured directly without regards to the volume of sample tested. This is the approach used at Bronson for highly lipemic samples.

Case Study: Pseudohyponatremia

Triglyceride Normal: <150 mg/dl	Sodium (indirect ISE) Normal: 135-145 mmol/L	Sodium (direct ISE) Normal: 135-145 mmol/L
2830 mg/dl	120 mMol/L	139 mMol/L

A second condition affecting sodium levels in diabetics is marked hyperglycemia. When glucose levels become elevated, the extracellular fluid (ECF) osmolality rises

and exceeds that of the intracellular fluid (ICF). This is due to glucose penetrating cell membranes slowly in the absence of insulin, which causes the movement of water out of cells into the ECF. Serum Na⁺ concentration then falls in proportion to the dilution of the ECF. This condition has been called translational hyponatremia because no net change in total body water (TBW) has occurred. No therapy directed specifically at the sodium level is indicated because Na⁺ concentration will return to normal once the plasma glucose concentration is lowered.

A correction factor may be applied to estimate what the serum sodium value would be for a given sample on a patient if their glucose level was normal. The sodium value reported by the lab is not due to analytical error. If used, correction factors are generally calculated by the clinician. The commonly applied calculation is to add 1.6 mMol/L to the reported sodium value for every 100 mg/dl of glucose over the normal range.² A study by Hillier TA, et al. demonstrated that this factor worked well up to glucose levels of 400 mg/dl.³ However, for values greater than 400 mg/dl, a correction factor of 4 was better. The study concluded with the recommendation to use a factor of 2.4 instead of 1.6.

Case Study: Translational Hyponatremia

Glucose Normal: 70-99 mg/dl	Sodium Normal: 135-145 mMol/L	Sodium Correction w/ factor of 2.4 per 100 mg/dl glucose above normal range
817 mg/dl	117 mMol/L	117+(7*2.4) = 134 mMol/L

It is possible that one patient may present with both hyperlipidemia and hyperglycemia, as well as diabetic ketoacidosis (DKA), an additional factor impacting Na⁺ measurements. In DKA, the increased acidity caused by increased ketone body production is reflected by a compensatory decrease in plasma bicarbonate and sometimes chloride. Plasma Na⁺ decreases because of the associated polyuria and co-excretion of these cations with acetoacetate and Beta-hydroxybutyrate.

References:

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(continued) 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.

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