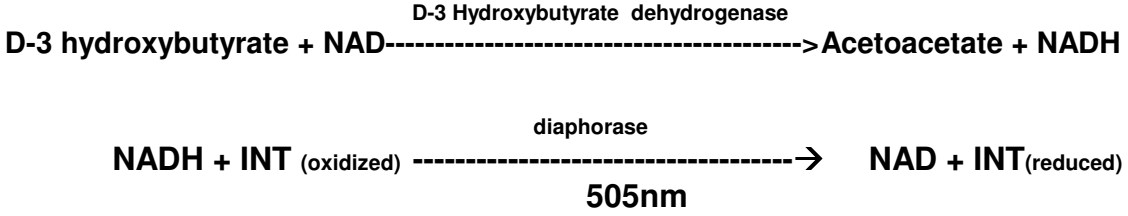
Beta-Hydroxybutyrate
Stanbio LiquiColor Reagent
Vista 1500

Principle
Enzymatic quantitation of β-hydroxybutyrate by β-hydroxybutyrate dehydrogenase has been reported. In the Stanbio method, β-hydroxybutyrate (D-3-hydroxybutyrate) in the presence of NAD gets converted to acetoacetate and NADH at pH 8.5 by β-hydroxybutyrate Dehydrogenase (D-3-hydroxybutyrate Dehydrogenase). At this pH, the reaction is favored to the right. The NADH produced reacts with INT in the presence of diaphorase to produce color at 505nm.



Clinical Significance
Ketosis is a common feature in acutely ill patients. In subjects suffering from starvation, acute alcohol abuse, or diabetes mellitus, ketosis can result in severe life threatening metabolic acidosis. The presence and degree of ketosis can be determined by measuring blood levels of β-hydroxybutyrate.

Ordinarily, β-hydroxybutyrate is the ketoacid present in the greatest amount in serum. It accounts for approximately 75% of the ketone bodies which also contain acetoacetate and acetone. During periods of ketosis, β-hydroxybutyrate increases even more than the other two ketoacids, acetoacetate and acetone, and has been shown to be a better index of ketoacidosis including the detection of subclinical ketosis.

In diabetics, the measurement of β-hydroxybutyrate as well as the blood glucose is needed for the assessment of the severity of diabetic coma and is essential for the exclusion of hyperosmolar non-ketotic diabetic coma. Moreover, the insulin requirements are often based on the extent of the existing hyperketonemia shown by the blood levels of β-hydroxybutyrate is therefore extremely important in the assessment of ketosis.

Specimen

Test # 14014, 'BUTYRATE'

Specimen Type	**serum**, **plasma** (heparinized, EDTA, sodium fluoride)
Preferred Volume	0.5 mL preferred, minimum of 150µL
Storage/Stability	Serum and Plasma 7 days at 2-8℃

Reagents/Materials

B-Hydroxybutyrate LiquiColor® Kit	
Enzyme (R1) Cat No. 2441, 50mL Contents: B-hydroxybutyrate dehydrogenase and diaphorase enzymes	Storage: 2-8℃ Stability: Until marked expiration date on respective labeling. Onboard Stability: 30 days Preparation: Ready to Use, R2-Protect from light. Precautions: For in vitro diagnostic use only. Avoid skin contact with reagents. If this occurs wash immediately with water.
Catalyst (R2) Cat No. 2442, 8.5mL Contents: NAD, INT, and oxalate	
Standard, 1mmol/L, Cat. No. 2443, 3mL Contents: 1mM Sodium D-3-hydroxybutyrate	
Multi 2 Sample Diluent, KD694:	Storage: 2-8℃ Stability: Until marked expiration date on respective labeling. Onboard Stability: 30 days Preparation: Ready to Use

Instrumentation/Equipment

Dimension Vista 1500
EMPTY Flex® 12-well, Cat.No. S999
3.0mL syringe, qty 2
18g x 1.5 inch blunt needle device, BD 305180, qty 2
Vista sample cups
Blue Vista Rack
Vista empty QC vials and caps

Quality Control

Material	Stanbio β-Hydroxybutyrate/TDM Bi-Level Controls. Assayed Controls Low and High, Ref No 2465-605, 5mL each
Stability:	Unopened vials are stable until marked expiration date at 2-8℃. Open vials are stable 60 days at 2-8℃, Onboard stability is 15 days. Discard if turbidity or any change in appearance occurs.
Preparation:	Gently mix the control solution by inversion. Ready for use.

	Dispense 2400 uL of control material into Vista Vials.
Frequency:	Both levels, once each day of patient testing. When opening a new box of reagent. Whenever there is reason to suspect issues with assay. Following calibration.

Warning and Precautions

1. *As with all chemical reagents, contact with skin should be avoided.*
2. *Handle blood specimens as potentially infectious samples and follow the guidelines established by the Centers of Disease Control (CDC) Atlanta, GA, for blood collection and handling (Document 20 CFR 1919.1030).*

Procedure

Test # 14014, 'BUTYRATE'.

Test Conditions

Sample Volume	2.8 µL, chase 2µL
Reagent 1 Volume	100 µL, chase 10 µL
Reagent 2 Volume	15µL, chase 7 µL
Reaction Time	7.03 minutes
Wavelength	510 nm
Type of Measurement	Rate

Preparing & Loading Reagent

1. With blunt needle, pierce a vent hole into one corner of film over each well to be filled, do not dispense reagent yet. This should be done to wells 1-5 and well 10.
2. Gently mix reagent bottle by swirling. Avoid formation of foam or bubbles.
3. Load R1 Enzyme Reagent into Wells 1-5 of EMPTY Flex by utilizing syringe with attached 18g blunt needle. Dispense 3.0 mL into each well 1-5, insert needle into corner of film avoiding center (opposite of corner which was pierced with vent hole).
4. Load 2.5 mL of R2 Catalyst Reagent into Well 10 with new syringe/needle.

5. Do not remove the clear film or attempt to reseal the punctures on the wells.
6. Document Reagent as XBHB, and new 30 day expiration on outside of flex, along with your initials. *Do not write over barcode*.
7. Place the reagent cartridge in the reagent load area of analyzer and press the Load button.
8. Press the Advanced icon, then the Inventories icon.
9. Select the Reagent Inventory from the menu. It may take a couple minutes for the new flex to appear in the inventory screen.
10. Click on the line with EMPTY in the Name column.
11. From the box that appears, select the mnemonic XBHB.
12. Enter the lot number in the Lot Field (as shown on reagent box), or choose Create New Lot.
13. In the second Lot Expiration field, type the reagent expiration date, as shown on the box.
14. Press Finish to accept the data.

Calibration

Frequency: Once every 30 days, for each new lot of reagent, after major maintenance or service to analyzer, as needed for troubleshooting quality control results.
Calibration Material: Standard, 1mmol/L, Cat. No. 2443
A 3-point calibration is performed using onboard diluent and the provided standard.

The standard and two dilutions (x2 and x10) act as the three points.

1. Setup Calibration for each *new lot* of calibrator:
    a. Press Advanced, Calibration.
    b. Select Calibrators from the menu.
    c. Select New from bottom of screen.
    d. Enter calibrator name BHB Standard
    e. Confirm Open Channel field states YES.
    f. Enter Calibrator Lot Number from vial.
    g. Enter expiration date, as shown on bottle.
    h. Select onboard stability as 30 days.
    i. Select open vial stability as 30 days.
    j. Enter maximum puncture as 99.
    k. Enter fluid type: calibrator.
    l. Select the test XBHB under methods.
    m. Adjust volume to 500uL.
    n. Enter bottle value under the bottle value selection: A as 1.0mM
    o. Enter Calibration Point Values. (Level 1 as 0.1mM, Level 2 as 0.5mM, Level 3 as 1.0 mM).
    p. Select Save Changes to finalize Calibrator.

2. Setup Quality Control for each *new lot*:
    a. Press Advanced, QC
    b. Choose QC Product Setup.
    c. Highlight the BHB Control currently in use.
    d. Choose Add Parallel Lot
    e. Enter Lot Number from vials, bottle Expiration for each level.
    f. Catalog numbers should be entered as BHB Low and BHB High.
    g. Choose Save Changes.
    h. Enter QC Product Setup, Edit Ranges
    i. Select each Control level, enter range from package insert into field.
    j. Once all levels entered, press Switch Parallel Lots.
    k. Choose to switch and inactivate current lot.

3. Order Calibration (with new lot and every 30 days as patient testing indicates).
    a. Advanced, Calibration
    b. Calibrate by Lot
    c. Select XBHB method
    d. Order Calibration
    e. Confirm the use vials box is checked.
    f. Scan blue rack.
    g. Press OK.

4. Load Samples
    a. For Calibration: Cup 1 place 500uL of 1.0mM Standard
    b. Place rack on load lane.

5. Once calibration is complete, Order QC
    a. Press QC tab
    b. Order QC
    c. Select Run QC Panel
    d. Select QC Material: BHB Assayed Control
    e. Check off Level 1 and Level 3
    f. Select QC Source: vials
    g. Submit
    h. Confirm run lists at bottom, right of screen.

6. If QC is within range, continue with patient testing. If QC is not found within range, troubleshoot assay as shown below, until found acceptable:
    a. Confirm all reagents, calibrator, and controls are within expiration date.
    b. Rerun QC with freshly prepared Vista QC vials
    c. Recalibrate with fresh cup of standard
    d. Prepare new EMPTY flex and recalibrate
    e. Try new reagent lot and recalibrate

Reporting Results

Reference Range: **0.02 mM/L – 0.27 mM/L**
Analytical Measurement Range: **0 – 4.0 mM/L**
Clinical Reportable Range: **0 – 20.0 mM/L**

1. Results may be reported in LIS by using Results Entry.
2. Results that are zero (0) mM/L should be reported as zero (0) mM (millimole/liter)
3. Dilute samples with values greater than 4.0 mM/L. Results obtained > 4.0 mM may be autodiluted by the Vista, using a x5 factor dilution (35µL of specimen).
4. After dilution, samples achieving values outside the analytical range should be reported as >20.00mM/L in LIS.

Performance Characteristics From MMCI In-house performed studies.

Linearity: The procedure described is linear to 4.0 mM β-hydroxybutyrate.
Sensitivity: Concentration of β-hydroxybutyrate of 0.18 can be clearly distinguished at the 95% confidence limit, confirming manufacturer's claim.
Precision: Precision studies were conducted using two control pools containing 0.18mM and 3.80 mM β-hydroxybutyrate.

	N	Mean mM	SD mM	CV (%)
Within Day	21	0.1879	0.0041	2.2

	18	3.7916	0.0515	1.4
Between Day	23	0.1861	0.0056	3.0
	14	3.7983	0.0230	0.6

Interfering Substances (Stanbio Package insert claims):

1. No significant changes in values were observed when the following analytes were added to serum containing 0.5mM β-hydroxybutyrate:

Analyte	% recovery
Glucose 2000 mg/dL	96
Acetoacetic acid 5mM	96
Creatinine 5 mg/dL	106
Ascorbate 3 mg/dL	106
Bilirubin 10 mg/dL	96
Uric Acid 16 mg/dL	102
Triglycerides 417mg/dL	104
Cholesterol 314 mg/dL	94
Lactic Dehydrogenase 1515 U/mL	93
Sodium lactate 96mg/dL	99

2. Hemolysis showing an OD at 540nm of 2.0 was added to test and found not to interfere.
3. Lactic Dehydrogenase and lactate have been shown to interfere with the assay. The incorporation of oxalic acid in this reagent eliminates this interference as reported.

References

1. Stanbio β-Hydroxybutyrate LiquiColor Procedure No. 2440 CE IFU, Stanbio Laboratory, Boerne, Texas. DN RBR.2440.CE.00, 06/2004.

REVISION HISTORY			
Rev	**Description of Change**	**Author**	**Effective Date**
0.0	Initial release	T King	1/3/2013

Author: Theresa King MLS(ASCP) 1/3/2013

Medical Director Approval: Devendra V. Trivedi. 1/18/2013

Coordinator	Date	Medical Director	Date
Theresa R King	1/4/2013	Robert Omsicka, PhD	1/17/2013