Purpose

To provide instructions for the *in vitro* diagnostic measurement of β-Hydroxybutyrate level in serum or plasma.

Specimen

Required	Serum
Acceptable	Plasma collected with Sodium or Lithium Heparin
Volume	100 microliter (µL) (minimum 40 µL) or as required
Container	Red top or Green top
Special Handling	Not applicable
Stability and Storage	• For 24 hours at 20 – 25ºC • For 5 days at 2 – 8ºC

Reagents

Stanbio Laboratory β-hydroxybutyrate LiquiColor® Procedure No. 2440

Material	Unopened Stability	Opened/Onboard Stability
Enzyme (R1) (β-hydroxybutyrate dehydrogenase and diaphorase enzymes)	Until expiration date at 2 - 8°C	For 14 days at 2 – 8°C
Catalyst (R2) (NAD, INT, and oxalate)	Until expiration date at 2 - 8°C	For 14 days at 2 – 8°C

Supplies and Equipment

- Empty Flex™ Reagent Cartridge
- Automatic Pipettes and tips
- Short Sample Cups (SSC)
- Sample Segments

Calibration

Material	Unopened Stability	Opened/Onboard Stability
Stanbio Laboratory β-hydroxybutyrate LiquiColor® Calibration Standard (1.0 millimols/liter (mmol/L) Sodium D-3-hydroxybutyrate)	Until expiration date at 2 – 8°C	• 1.0 mmol/L Standard until expiration date at 2 -8ºC • Prepared Standards use immediately
Frequency	Calibration must be performed • Every 14 days for any one lot number • Every new reagent kit / lot number • As determined by the Quality Control	
Procedure	• β-Hydroxybutyrate is a Linear Calibration method • To reset the Coefficients for any one lot number use C0 = 0.0 and C1 = 1.0	

STEP	ACTION
1.	Prepare Calibrators Prepare 0.1 and 0.5 mmol/L standards from the Calibration Reagent Standard (1.0 mm/L) provided in the kit. ***0.1 Standard:*** 20 microliters (µL) of 1.0 Standard plus 180 µL Distilled H_2O ***0.5 Standard:*** 100 µL of 1.0 Standard plus 100 µL Distilled H_2O ***1.0 Standard:*** 200 µL
3.	To program the calibration, from the Operating Menu, press **F5: Process Control**
4.	Select **F1: Calibration** and press the "enter" key for the password
5.	Press **F2: Setup and Run**
6.	Select **BOH** by pressing the Control and Na/K keys Press **F1: Other lot** to select the appropriate lot number to be calibrated
7.	Complete the information on the screen: • **Operator ID:** enter your initials • **Calibrator Name/Lot #:** enter BH followed by the Standard Lot # • **Segment Position:** enter the letter of the segment and the start position for the calibration (Example: A1) • **Calibrator Bottle Values:** enter 0.1, 0.5, and 1.0
8.	Press **F8: QC yes/no** to add QC
9.	Press **F4: Assign Cups,** then **F7: Load/Run**
10.	Verify that there is adequate volume, load segment, and press **F4: Run**
11.	To review data after calibration is completed: • From the Operating Menu, press: **F5: Process Control** • Press **F1: Calibration,** then hit the "enter" key for the password • Press **F3: Review Data** • Select **BOH**
12.	Press **F7: Calculate**

<table>
<tr><td></td><td colspan="2"></td></tr>
<tr><td rowspan="3">13.</td><td colspan="2">Scan for outliers within the replicates for each level of calibrator
NOTE: “Assay range” codes on the print out does not mean that the calibration needs to be rejected</td></tr>
<tr><td>**IF**</td><td>**THEN**</td></tr>
<tr><td>There is an outlier within the replicates</td><td>• Press **F8: Reject Data** to reject the calibration
• Repeat the calibration</td></tr>
<tr><td></td><td>The precision is good with no outliers</td><td>Press **F6: See QC** to review the QC, Slope (m), Intercept (b), and Correlation Coefficient (r) with the calculated calibration</td></tr>
<tr><td rowspan="4">14.</td><td colspan="2">After QC, Slope, Intercept, and Correlation Coefficient review</td></tr>
<tr><td>**IF**</td><td>**THEN**</td></tr>
<tr><td>• Slope(m): For the Linear calibration is <0.97 or >1.03
• Correlation coefficient is <0.990 or >1.000</td><td>• Press **F8: Reject Data** to reject the calibration
• Verify that the programmed calibrator lot number and the bottle values are correct
• Repeat the calibration</td></tr>
<tr><td>• Slope(m): For the Linear calibration is 0.97-1.003
• Intercept (b): close to zero or clinically insignificant
• Correlation coefficient(r): 0.990-1.000
• QC is within the established range</td><td>Press **F2: Accept Data** to accept the calibration</td></tr>
<tr><td></td><td>The calibration is acceptable, and the QC is not within range</td><td>Troubleshoot Controls</td></tr>
</table>

Quality Control (QC) Specifications

Material	Unopened Stability	Opened/Onboard Stability
Stanbio Laboratory TDM/ β-Hydroxybutyrate Tri-Level Controls	Until expiration date at 2 - 8°C	For 30 days at 2 – 8°C
Frequency And Levels	Three levels of controls must be run • Every 24 hours • After loading a new Flex™ reagent cartridge • After calibration • After any major maintenance/ repairs have been performed on the analyzer	

Procedure A
User Defined Method Identification

A. Method Specifications
The following specifications are programmed into the Dade Dimension RxL in the specified field under Diagnostics (F7) and Open Channels (F8).

Channel # __ Name: XBOH **Measurement Mode:** Absorbance **Standard Curve:** Linear

Delivery	Time	Component 1	Component 2	Component 3	Chase	Mix
1st R	-57.6	(A) 360 µL	0	0	0 µL	NONE
2nd R	61.6	(B) 53 µL	0	0	20 µL	STRONG
3rd R	***	( ) 0 µL	0	0	0 µL	NONE
S1		10 µL	0	0	10 µL	STRONG

Photometry	Time	Cartridge Configuration	Well 1	Well 2	Well 3	Well 4	Well 5	Well 6
P1:	30.0	Component:	(R1)	(R1)	(R1)	( )	(R2)	( )
P2:	390.0	Aliquots:	10	10	10	0	30	0
P3:	***	#Well Life [hours]:	336	336	336	336	336	336
P4:	***	#On Board Life: 336 hrs (14 days)			#Calibration: 336 hrs (14 days)			
Sample Volume:		10 µL						

B. Method Calculation
The following specifications are programmed into the Dade Dimension in the specified field under Diagnostics (F7), Open Channels (F8), and Calculations (F7).

{A = BICH (P1, 510 NM, 700 NM);
B = BICH (P2, 510 NM, 700 NM);

C = B*(1.120) – A*(1.120);

RETURN C;}

Procedure B
Flex™ Reagent Cartridge Preparation

STEP	ACTION
1.	Label the outside of the kit with following information • Expiration date • Flex Lot number (see step # 2 for explanation) • Sequence numbers of the Flexes 1, 2, 3, and 4 as they are prepared (see step # 3 for explanation)
2.	Lot number is determined by the expiration date and month of the unopened kit using the Julian Calendar

	For example, the kit is β-Hydroxybutrate and it expires December 30, 2008. The lot number would be BH8365 • BH is the mnemonic for β-Hydroxybutrate • First number, 8 is the expiration year (2008) • Last three numbers, 365 is the Julian calendar date (December 30th)
3.	Sequence number is determined by the number of Flexes per kit and the expiration date of each newly prepared Flex There are 4 Flexes per kit (120 tests) For example, there are 4 Flexes per kit and the first Flex was prepared 3/13/2007. The Flex is stable for 2 weeks. Therefore, the expiration date will be 3/27/2007. The Sequence number will be 10320. Second Flex will start with 2, Third with 3, and Forth with 4 followed by the expiration date **NOTE:** • The first number (1) is the first Flex of the kit • The last 4 numbers are the month and day of that prepared Flex expires
4.	Obtain an empty Flex reagent cartridge (see attachment A)
5.	Label the empty Flex with the Lot number and Sequence number
6.	Pierce wells 1, 2, 3 and 5 in a corner using a pipette tip
7.	Add 4 mL of R1 in wells 1, 2, and 3 of the empty compartment Flex
8.	Add 2 mL of R2 in well number 5 of the empty compartment Flex
9.	Load Flex (follow procedure C below)

Procedure C

Loading of Flex™ Reagent Cartridge

STEP	ACTION
1.	Press **ALT I** (Inventory Screen)
2.	Press **F4: Add Reagent**
3.	Place the β-Hydroxybutrate flex in the automatic loader **NOTE:** Do not use the RMS autoloader
4.	When prompted , use the keyboard to enter the Flex cartridge information
5.	At **METHOD:** Press delete twice to remove the default method Enter the **XBOH** (X indicates it is an open channel method) then press Enter
6.	At **LOT NUMBER:** Press delete to remove the default lot number Enter the lot number as specified in Procedure B above then press Enter
7.	At **SEQUENCE NUMBER:** Press delete to remove the default sequence number Enter the sequence number as specified in Procedure B above then press Enter
8.	Press **F1: ACCEPT**
9.	Allow the reagent to equilibrate for 15 – 30 minutes on the analyzer before use

Procedure D
Analysis Procedure

NOTE: Refer to the Dade Dimension® *Clinical Chemistry System Operator's Guide* (page 2-1 to 2-57) for step by step instructions for loading and processing samples and basic operation of the instrument.

STEP	ACTION	
1.	Check analyzer daily maintenance	
	IF	**THEN**
	Routine maintenance has been performed	Proceed to step # 2
	Routine maintenance has not been performed	Perform maintenance as described in the Dade Dimension® Operation Guide
2.	Check reagent inventory	
	IF	**THEN**
	Reagent inventory sufficient	Proceed to step # 3
	Reagents are insufficient	Prepare a new Flex™ reagent cartridge **NOTE:** Calibration is required when opening a new reagent kit
3.	Check Quality Control status	
	IF	**THEN**
	Quality Control has been performed within the last 24 hours	Proceed to step # 5
	Quality Control has not been performed within the last 24 hours	Proceed to step # 4
4.	Run Quality Control	
	IF	**THEN**
	Quality Control within range	Proceed to step # 5
	Quality Control out of acceptable range	Follow Troubleshooting controls
5.	Load sample rack	
6.	Press RUN on the keyboard	
	Determine if the result is within the Analytical Measurement Range (AMR) and the Clinically Reportable Range (CRR)	
	IF	**THEN**
	Result is < AMR	Report as < AMR
	Result is > AMR	Dilute the sample before reporting • Analyzer automatically dilutes the sample and calculates the results, or • Make appropriate dilution when result is above the auto dilution range **NOTE:** A manual dilution supersedes an auto dilution

	Result is > CRR	Result as > CRR

NOTE: Refer to Dade Dimension® *Clinical Chemistry System* Appendix A – Understanding Test Report Messages (page A-13 to A-17) to resolve any test report error messages.

Calculations

- For auto calculation of results, enter the dilution factor in the dilution field in Enter Sample Data Screen
- Manually calculate diluted samples by multiplying the result by the dilution factor

Method Limitations

Dilutions	**RANGE OF DILUTION**	**DILUENT**
	When result is >AMR, analyzer automatically dilutes the sample and calculates the result or make appropriate dilution	Distilled Water (H_2O)
Interfering Substances	See reagent package insert for possible interfering substances	

Resulting

Units	mmol/L (Result to one decimal place)
Expected	• Non-fasting (random) <0.4 mmol/L • Fasting 0.2 – 2.8 mmol/L
Critical	User Defined

Principle

Ketone bodies or ketoacids are breakdown products of fat that can accumulate in the blood as a result of inadequate insulin levels (diabetes mellitus), inadequate calorie intake (starvation) or other disorders that interfere with carbohydrate metabolism.

Ordinarily, β-hydroxybutyrate is the ketoacid present in the greatest amount in serum. It accounts for approximately 75% of the ketone bodies which also include 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.

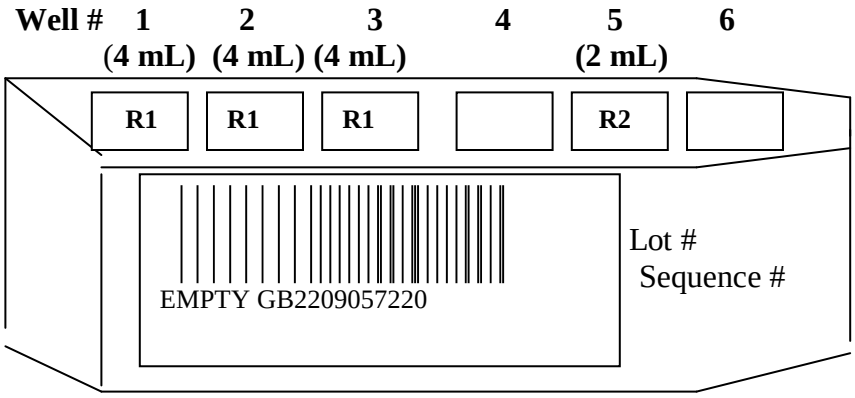
In diabetics, the measurement of β-hydroxybutyrate as well as the blood glucose is needed for assessment of the severity of diabetic coma and is essential for the exclusion of hyperosmolar non-ketotic diabetic coma. Moreover, the insulin requirements are often based on the extent of the existing hyperketonemia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

The assay for β-hydroxybutyrate on the Dade Dimension RxL is a two enzymatic process. First, β-hydroxybutyrate (D-3-hydroxybutyrate) in the presence of NAD is converted to acetoacetate and NADH at pH 8.5 by β-hydroxybutyrate dehydrogenase (D-3-hydroxybutyrate dehydrogenase). In the second step, the NADH produced reacts with INT in the presence of diaphorase to produce color which is read by the analyzer at 510 nm. The amount of color produced is proportional to the amount of β-hydroxybutyrate in the sample.

$$\text{D-3-Hydroxybutyrate} + \text{NAD} \xrightarrow{\text{D-3-Hydroxybutrate dehydrogenase}} \text{Acetoacetate} + \text{NADH} + H^{+}$$

$$\text{NADH} + \text{INT (oxidized)} \xrightarrow{\text{Diphorase}} \text{NAD} + \text{INT (reduced)}$$

Attachment A: Empty Flex™ Reagent Cartridge



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