DXC (BOHB) BETA-HYDROXYBUTYRATE

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PURPOSE

To provide instructions for the quantitative determination of Beta-Hydroxybutyrate on the DXC 600/800.

PRINCIPLE

BOHB reagent, when used in conjunction with UniCel® DxC 600/800 System(s), is intended for the quantitative determination of B-Hydroxybutyrate, the ketoacid present in the greatest amount in serum.

BACKGROUND

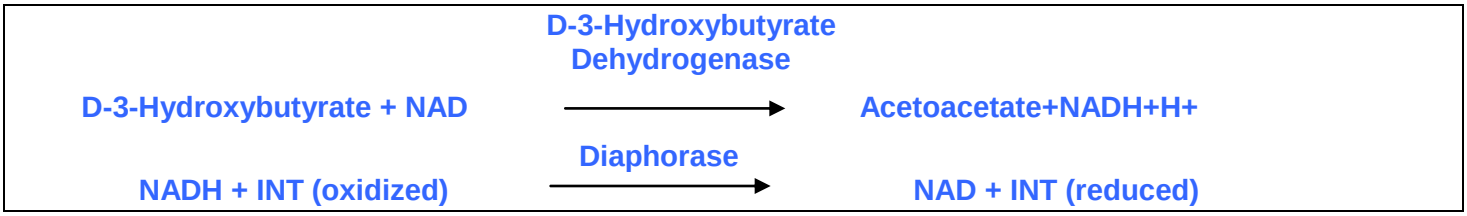
Clinical Significance

Beta-Hydroxybutyrate measurements are used in the diagnosis and treatment of ketosis which can result in severe life threatening metabolic acidosis. Ordinarily, B-Hydroxybutyrate is the ketoacid present in the greatest amount in serum, accounting for approximately 75% of the ketone bodies. During periods of ketosis, B-Hydroxybutyrate increases even more than the other two ketoacids, acetoacetate and acetone, showing it to be a better index of ketoacidosis, including the detection of subclinical ketosis.

Methodology

BOHB reagent uses the enzymatic quantitation of B-hydroxybutyrate by B-hydroxybutyrate dehydrogenase. In the Stanbio method, B-hydroxybutyrate (D-3-hydroxybutyrate) in the presence of NAD gets converted to acetoacetate and NADH at the pH 8.5 by B-hydroxybutyrate dehydrogenase (D-3hydroxybutyrate 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 505 nm.

Chemical Reaction



RELATED DOCUMENTS

R-PO-CH-0810	Quality Control Program General Laboratory
R-PO-CH-0809	Quality Control Westgard Rules Statistics
R-PR-AD-0540	Specimen Rejection/Cancellation Protocol
J-F-CH-0820	DXC 800 Controls
J-F-CH-0826	DXC 800 Calibrators
J-F-CH-1940	DXC Analytical Measurement Range
M-F-CH-0820	Chemistry ControlsM-F-CH-0826 Chemistry Calibrators

SPECIMEN

Type of Specimen

Biological fluid samples should be collected in the same manner routinely used for any laboratory test. Freshly drawn serum or plasma are the specimens of choice. Acceptable anticoagulants are listed in PROCEDURAL NOTES section of this chemistry information sheet. Whole blood is not recommended for use as a sample.

Specimen Storage and Stability

1. Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum or plasma be physically separated from contact with cells within two hours from the time of collection.

2. Separated serum or plasma should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C for up to 1 week. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.

Sample Type	Volume	Sample Stability
Plasma/Serum EDTA, heparin or sodium fluoride	0.5mL	• Separate serum from cells within 2 hours • Room Temp 8 hours • Refrigerated 1 week • Frozen 1 months

Criteria for Unacceptable Specimens

Refer to the PROCEDURAL NOTES section of this chemistry information sheet for information on unacceptable specimens.

Sample Volume

A filled 0.5 mL sample cup is the optimum volume. For optimum primary sample tube volumes in primary tube samples and minimum volumes, refer to the Primary Tube Sample Template for your system

REAGENTS

Contents

Each kit from Stanbio Laboratory contains the following items:

Reactive Ingredients	
B-hydroxybutyrate, dehydrogenase and diaphorase enzymes (R1 Enzyme)	50 mL
NAD, INT, and oxalate (R2 Catalyst)	8.5 mL
Sodium D-3-hydroxybutyrate 1mM (Standard 1mmol/L)	3 mL

Volume per Test	
Sample Volume	3 uL
Total Reagent Volume	240uL
Cartridge Volumes	A 25mL B -- C 4.2mL

Reagent Preparation

25 mL of Reagent R1 is pipetted into "A" compartment and 4.2 mL of Reagent R2 into "C" compartment of the user defined cartridge. Each reagent kit contains enough for 2 user defined cartridges.

Acceptable Reagent Performance

The acceptability of a reagent is determined by ensuring that quality control results are within acceptance criteria.

Reagent Storage and Stability

BOHB reagent, when stored unopened at +2°C to +8°C, will remain stable until the expiration date printed on the label. Once opened, the reagent is stable for at least 14 days onboard the analyzer. Usage beyond 14 days should be monitored by Quality Control recovery. Recalibration or new reagent may be necessary if Quality Control indicates. Contamination must be avoided. Do not use beyond the manufacturer's expiration date.

Calibrator Required

1.0 mmol/L Standard comes in the kit and is used to calibrate the assay.
Deionized H_2O is used as the low calibrator.

Calibrator Preparation

No preparation is required.

Calibrator Storage and Stability

1.0 mmol/L Standard may be stored at +2°C to +8°C until the expiration date on bottle.

Calibrator Information

1. The system must have a valid calibration factor in memory before control or patient samples can be run.

2. Under typical operating conditions the BOHB reagent cartridge must be calibrated every 14 days and also with certain parts replacement or maintenance procedures, as defined in the UniCel DxC 600/800 System *Instructions For Use* (IFU) manual.

3. For detailed calibration instructions, refer to the UniCel DxC 600/800 System *Instructions for Use* (IFU) manual.

4. The system will automatically perform checks on the calibration and produce data at the end of calibration. In the event of a failed calibration, the data will be printed with error codes and the system will alert the operator of the failure. For information on error codes, refer to the UniCel DxC 600/800 System *Instructions for Use* (IFU) manual.

QUALITY CONTROL

Stanbio Laboratory TDM/B-Hydroxybutyrate Controls, Low, Medium and High. Controls may be stored at +2°C to +8°C until the expiration date on the bottle. Control solution is stable for 60 days in the refrigerator after opening. Discard if turbidity or any change in appearance occurs.

See Related Documents J-F-CH-0820 DXC 800 Controls & M-F-CH-0820 Chemistry Controls for QC frequency

STEPS

1. If necessary, load the reagent onto the system.

2. After reagent load is completed, calibration may be required.

3. Program controls for analysis.

4. After loading controls onto the system, follow the protocols for system operation. To load samples manually refer to the FHS DXC Series Manual Sample Programming procedure. For detailed testing procedures, refer to the UniCel DxC 600/800 System *Instructions for Use* (IFU) manual.

CALCULATIONS

SYNCHRON® System(s) perform all calculations internally to produce the final reported result. The system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming

REFERENCE RANGE

Sample Type	Range
Serum/ Plasma	0.0 – 0.29 mmol/L

Analytic Range

The SYNCHRON® System(s) method for the determination of this analyte provides the following analytical range:

Sample Type	Conventional Units (BOHB)
Serum or Plasma	0.0 – 4.5 mmol/L

The low end of the analytical range represents the minimum level of detection. Sample values greater than 4.5 mmol/L should be diluted with deionized water and reanalyzed.

Reporting results outside of analytical range

Lower limit of detection	0.1 mmol/L	Results <0.1: Report as <0.1 mmol/L
Upper limit of range	4.5 mmol/L	Results >4.5 should be diluted with deionized H_20 starting at X2, reanalyzed and dilution factor applied. The maximum allowable dilution is X5. Results >22.5 are reported as >22.5 mmol/L.

Sensitivity

Concentrations of B-hydroxybutyrate of 0.18, 0.28, and 0.38mM (1.8, 2.9 and 3.9 mg/dL) can be distinguished at the 99.9% confidence limit.

LIMITATIONS

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.

INTERFERENCES

1. The following substances were tested for interference with this methodology: No significant changes in values were observed with the following analytes when added to serum containing 0.5mmol/L (5.2mg/dL) B-Hydroxybutyrate:

Substance	Source	Level Tested	Observed Effect
Bilirubin	Porcine	10 mg/dL	No significant interference. 96% recovery
Triglycerides	Human	417 mg/dL	No significant interference. 104% recovery
Cholesterol	Human	314 mg/dL	No significant interference. 94% recovery

In addition, hemolyzed serum with an OD at 540nm of 2.0 was added to the test and found not to interfere**.**

REFERENCES

Package insert for B-Hydroxybutyrate LiquiColor Procedure No. 240 Stanbio Laboratory Revision 6/04

Package insert for Tri-Level TDM/B-Hyrodxybutyrate Controls, Ref No. 2460, Stanbio Laboratory, Revision 12/07/12

Synchron DxC Clinical System User-Defined Chemistry Setup, B-Hydroxybutyrate Liquicolor, Revision 02.12 Nov 2010/4

DOCUMENT APPROVAL **Purpose of Document / Reason for Change:**			
1. Updated open storage and stability of reagent and QC material 2. Added Low Calibrator information 3. Added References 4. Added dilution info to Reporting Results outside of analytical range section 5. Changed lower limit of detection from 0.0 to <0.1			
Committee Approval Date	☒ Date: 7/2/15 ☐ NA – revision of department-specific document which is used at only one facility	**Medical Director Approval** *(Electronic Signature)*	Katie Wilkinson, MD 7/30/15