University of California, Davis Health System
Department of Pathology and Laboratory Medicine
Automated Chemistry/Urinalysis

Beta-Hydroxybutyrate (BHB) - Serum, Plasma **Technical Procedure 3108**
Beckman UniCel DxC Systems

Prepared By	Date Adopted	Supersedes Procedure #
Michael Inn	July 24, 2012	Ketone Bodies 3422

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Beta-Hydroxybutyrate (BHB) - Serum, Plasma
Beckman UniCel DxC Systems

Technical Procedure 3108

For *In Vitro* Diagnostic Use Only

Principle

Intended Use

Beta-hydroxybutyrate (BHB) reagent, when used in conjunction with UniCel® DxC 800 System(s) and Stanbio Laboratory Standard (Calibrator), is intended for quantitative determination of Beta-hydroxybutyrate concentration in human serum or plasma.

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.(1) The presence and degree of ketosis can be determined by measuring blood levels of ß-hydroxybutyrate.

Ordinarily, ß-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.(2,3,4) During periods of ketosis, ß-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.(5,6,7,8)

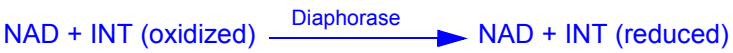
In diabetics, the measurement of ß-hydroxybutyrate as well as the blood glucose is needed for the assessment of the severity of diabetic coma and is essential for the exclusion of hyperosmolar non-ketotic diabetic coma. Moreover, the insulin requirements are often based on the extent of the existing hyperketonemia(9) shown by the blood levels of ß-hydroxybutyrate is therefore extremely important in the assessment of ketosis.

Methodology

Enzymatic quantitation of ß-hydroxybutyrate by ß-hydroxybutyrate dehydrogenase has been reported.(10,11,12) In the Stanbio method, ß-hydroxybutyrate (D-3-hydroxybutyrate) in the presence of NAD gets converted to acetoacetate and NADH at pH 8.5 by ß-hydroxybutyrate dehydrogenase (D-3-hydroxybutyrate dehydrogenase). At this pH, the reaction is favored to the right.(12) The NADH produced reacts with INT in the presence of diaphorase to produce color at 505 nm.

The SYNCHRON® DxC800 System (per user-defined parameters) automatically proportions the appropriate sample and reagent volumes into a cuvette. The ratio used is one part sample to 80 parts reagent. The system monitors the change in absorbance at 520 nanometers. This change in absorbance is directly proportional to the concentration of BHB in the sample and is used by the System to calculate and express BHB concentration.

Chemical Reaction Scheme



INT=iodonitrotetrazolium chloride

Specimen

Acceptable Sample Containers

13 x 75 PST BD tubes
PST BD microtainers
Optimum volume: 0.5 mL, Minimum volume: 0.1 mL

Type of Specimen

Biological fluid samples should be collected in the same manner routinely used for any laboratory test.(4) Freshly drawn serum or plasma (EDTA, heparin, or sodium fluoride) are the preferred specimens. Acceptable anticoagulants are listed in *Procedural Notes* section of this chemistry information sheet. Whole blood or urine are 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.(5)
2. Serum or plasma ß-hydroxybutyrate levels are stable at least one week if kept refrigerated at +2°C to +8°C.

Sample Volume

The optimum volume, when using a 0.5 mL sample cup, is 0.1 mL of sample. For optimum primary sample tube volumes and minimum volumes, refer to the *Primary Tube Sample Template*.

Criteria for Unacceptable Specimens

Refer to the *Procedural Notes* section of this chemistry information sheet for information on unacceptable specimens.

Reagents

Contents

Each kit (Stanbio Laboratory) contains the following items: **Kit Reorder # 2440-058**

Approximately 190 tests
R1: 1 x 50 mL (Cat No. 2441)
R2: 1 x 8.5 mL (Cat. No. 2442)
One preparation insert

Volumes per Test

Sample Volume	3 µL
Total Reagent Volume	240 µL
Cartridge Volumes	
A	200 µL
B	-----
C	40 µL

Reactive Ingredients

Reagent Constituents
- R1
  - Beta-hydroxybutyrate dehydrogenase
  - Diaphorase enzymes
- R2
  - NAD
  - INT
  - Oxalate

Precautions
For In Vitro Diagnostic Use Only. Avoid skin contact with the reagents. If this occurs wash immediately with water.

Materials Needed But Not Supplied With Reagent Kit

Deionized water (low level calibrator)
At least two levels of control material
User-defined Reagent (UDR) cartridge

Reagent Preparation

1. Reagents are supplied ready to use.
2. Pipet 25 mL reagent R1 into compartment A of a User-Defined Reagent (UDR) cartridge.
3. Pipet 4.25 mL reagent R2 into compartment C of a User-Defined Reagent (UDR) cartridge.
4. Write the name (mnemonic BHB) of the reagent, date prepared, kit lot number and initials on the UDR cartridge and document in reagent log.
5. The remaining reagents (R1 & R2) are pipetted into the UDR after the calibration expires (14 days) before recalibration of the BHB UDR.
6. When calibration times out again or when tests are used up, the UDR is then discarded and a new UDR will be used with a new BHB reagent kit.

Acceptable Reagent Performance

The acceptability of this reagent is determined by successful calibration and by ensuring that quality control results are within acceptance criteria, as defined in the *Quality Control* section of this procedure.

Reagent Storage and Stability

Reagents are stable stored at +2°C to +8°C until expiration date on their respective labeling. Once opened, reagent is stable on board for up to 30 days. Contamination must be avoided. **DO NOT FREEZE**.

Equipment

This test is performed on the Beckman UniCel DxC 800 systems; Beckman-Coulter, Brea, California. For technical assistance, call the Beckman-Coulter hotline: 1-800-854-3633.

Refer to the UniCel DxC 800 systems *Reference Manual* for detailed instructions.

Calibration

Calibrator Required

Stanbio Laboratory Standard (Calibrator) 1 x 3.0 mL (Cat No. 2443)
Contains 1 mmol/L Sodium D-3-hydroxybutyrate
Deionized water (low level calibrator)

Calibrator Preparation

No preparation is required.

Calibrator Storage and Stability

Stable stored at +2°C to +8°C until expiration date on label. Once opened, contamination must be avoided.

Calibration Information

1. The system must have a valid calibration in memory before controls or patient samples can be run.
2. Under typical operating conditions the BHB reagent UDR cartridge must be calibrated every 14 days and also with certain parts replacements or maintenance procedures, as defined in the UniCel DxC 800 Systems *Instructions for Use* (IFU) manual.
3. For detailed calibration instructions, refer to the UniCel DxC 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 800 System *Instructions For Use* (IFU) manual.

Quality Control

At least two levels of control material should be analyzed daily. In addition, these controls should be run with each new calibration, with each new reagent cartridge, and after specific maintenance or troubleshooting procedures as detailed in the UniCel DxC 800 System *Instructions For Use* manual. More frequent use of controls or the use of additional controls is left to the discretion of the user based on workload and workflow or laboratory accreditation requirements and applicable laws

The following controls should be prepared and used in accordance with the package inserts. Copies of these inserts can be found in the *Control Procedures* section of the Beckman UniCel DxC *Chemistry Information Manual*. Quality control results should be evaluated and handled with respect to the Clinical Chemistry Quality Control Procedure #3000.T. Controls are compiled statistically in the LIS and reagent lot changes are documented on DxC Reagent Log sheets.

Quality Control Material

CONTROL	Cat. No.	STORAGE
Low Control - Silver Crimp (5 mL)*	2461	+2°C to +8°C**
High Control - Red Crimp (5 mL)*	2463	+2°C to +8°C**

*The measured amounts of ß-Hydroxybutyrate is in a synthetic serum substitute (SeraSub™) matrix.
**Store unopened vials in refrigerator (+2^{o}C to +8^{o}C) until expiration date on label. Control solution is stable for 60 days in refrigerator (+2^{o}C to +8^{o}C) after opening. Discard if turbidity or any change in appearance occurs.

Cat. No. for a set of low and high controls is 2465.

Testing Procedure

1. If necessary prepare reagent as defined in the Reagent Preparation section of this chemistry information sheet and load the reagent onto the system.
2. After reagent load is completed, calibration is required.
3. Program samples and controls for analysis.
4. After loading samples and controls onto the system, follow the protocols for system operation.

For detailed testing procedures, refer to the UniCel DxC 800 System *Instructions For Use* (IFU) manual.

Calculations

UniCel DxC Systems 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.

If the dilution was programmed in the DataLink, the final calculated result from a dilution will not be calculated by the UniCel DxC system but by the DataLink.

Reporting Results

The quantitation of ß-hydroxybutyrate is important in cases of ketoacidosis. In studies of healthy individuals who had fasted for 12 hours before blood collection, the range of ß-hydroxybutyrate was found to be from 0.02 mmol/L (0.2 mg/dL) to 0.27 mmol/L (2.81 mg/dL).(4,5) Other ranges have also been reported.(13)

To obtain mg/dL, divide the value obtained in mmol/L by 0.096.

Reference Intervals

The following reference intervals were taken from literature and a study performed on SYNCHRON Systems.(7)

Reference Intervals

Intervals	Sample Type	Conventional Units	S.I Units
Stanbio Lab	Serum or Plasma	0.21 - 2.81 mg/dL	0.02 - 0.27 mmol/L
UCDMC	Serum or Plasma	0.21 - 2.81 mg/dL	0.02 - 0.27 mmol/L

Refer to References (8, 9, 10) for guidelines on establishing laboratory-specific reference intervals

Critical Values

None

Procedural Notes

Anticoagulant Test Results

1. If plasma is the sample of choice, the following anticoagulants were found to be compatible with this method.

Compatible Anticoagulants[a]

Anticoagulant
Sodium Heparin
Lithium Heparin
Ammonium Heparin
EDTA
Sodium Fluoride

Limitations

The incorporation of oxalic acid in this reagent eliminates interference from lactic dehydrogenase and lactate which normally interferes with this assay.(12)

Interferences

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

Interferences

Substance	Level Tested	% Recovery
Glucose	2000 mg/dL	96
Acetoacetic acid	5 mmol/L	96
Creatinine	5 mg/dL	106
Ascorbate	3 mg/dL	106
Bilirubin	10 mg/dL	96
Uric Acid	16 mg/dL	102
Triglycerides	417 mg/dL	104
Cholesterol	314 mg/dL	94
Lactic dehydrogenase	1515 U/mL	93
Sodium lactate	96 mg/dL	99

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

Performance Characteristics

Analytical Measurement Range

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

Analytical measurement Range (AMR)

Sample Type	Conventional Units	S.I. Units
Serum or Plasma	0.0 - 83.3 mg/dL	0.0 - 8.0 mmol/L

Clinical Reportable Range:

Clinical Reportable Range (CRR)

Sample Type	Conventional Units	S.I. Units
Serum or Plasma	0.0 - diluted result mg/dL	0.0 - diluted result mmol/L

Samples with concentrations below the AMR and CRR will be reported as "**None detected**".
Samples with concentrations greater than the AMR should be diluted with deionized water and reanalyzed.

If the dilution was programmed in the DataLink, the final calculated result from a dilution will not be calculated by the UniCel DxC system but by the DataLink.

Sensitivity

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

Precision

Precision studies were conducted using two serum pools containing 0.25 mmol/L (2.6 mg/dL) and 1.0 mmol/L (10.4 mg/dL) ß-hydroxybutyrate.

The following results are averages of eighteen determinations.

As determined by Stanbio Laboratory

Precision Values

Type of Precision	Sample Type	Mean	SD	%CV
		mmol/L	mmol/L	
Within-day	Serum Pool 1	0.29	0.005	1.7
	Serum Pool 2	1.09	0.015	1.4
Between day	Serum Pool 1	0.26	0.014	5.2
	Serum Pool 2	1.05	0.018	1.7

Precision established by StanBio Laboratory on a Beckman DxC analyzer

Type of Precision	Sample Type	Mean	SD	%CV
		mmol/L	mmol/L	
Within-run	Low Control - Silver Crimp (5 mL)	0.20	0.0	0.0
	Medium Control - Green Crimp (5 mL)	1.08	0.04	4.0
	High Control - Red Crimp (5 mL)	4.46	0.16	3.6

Precision established at UCDMC

DxC800-4427

Type of Precision	Sample Type	Mean	SD	%CV
		mmol/L	mmol/L	
Within-run	Low Control - Silver Crimp (5 mL)	0.20	0.00	0.0
	Medium Control - Green Crimp (5 mL)	1.20	0.00	0.0
	High Control - Red Crimp (5 mL)	4.63	0.06	1.2
Day to day	Low Control - Silver Crimp (5 mL)	0.20	0.00	0.0
	Medium Control - Green Crimp (5 mL)	1.14	0.05	4.4
	High Control - Red Crimp (5 mL)	4.55	0.14	3.1

DxC800-4449

Type of Precision	Sample Type	Mean	SD	%CV
		mmol/L	mmol/L	
Within-run	Low Control - Silver Crimp (5 mL)	0.20	0.00	0.0
	Medium Control - Green Crimp (5 mL)	1.10	0.00	0.0
	High Control - Red Crimp (5 mL)	4.50	0.05	1.1
Day to day	Low Control - Silver Crimp (5 mL)	0.20	0.00	0.0
	Medium Control - Green Crimp (5 mL)	1.15	0.05	4.5
	High Control - Red Crimp (5 mL)	4.52	0.07	1.5

Accuracy

Recovery of the StanBio assayed controls from a within-run precision run determined by StanBio and UCDMC is compared in the following table.

Type of Precision	Sample Type	Mean	SD	%CV
		mmol/L	mmol/L	
StanBio Within-run	Low Control - Silver Crimp (5 mL)	0.20	0.00	0.0
	Medium Control - Green Crimp (5 mL)	1.08	0.04	4.0
	High Control - Red Crimp (5 mL)	4.46	0.16	3.6
DxC800-4427 Within-run	Low Control - Silver Crimp (5 mL)	0.20	0.00	0.0
	Medium Control - Green Crimp (5 mL)	1.20	0.00	0.0
	High Control - Red Crimp (5 mL)	4.63	0.06	1.2
DxC800-4449 Within-run	Low Control - Silver Crimp (5 mL)	0.20	0.00	0.0
	Medium Control - Green Crimp (5 mL)	1.10	0.00	0.0
	High Control - Red Crimp (5 mL)	4.50	0.05	1.1

Linearity Recovery

Linearity verification was performed using StanBio Labs Beta-Hydroxybutyrate Linearity Standards (Cat. No. 2450-604) with concentrations of 0.0, 0.50, 1.00, 2.00, 4.00 & 8.00 mmol/L. These are typical recoveries for a DxC800.

DxC800-4427

Expected	Measured	Recovery (%)
0.00	0.00	100
0.50	0.60	120
1.00	1.15	115
2.00	2.25	112
4.00	4.25	106
8.00	8.50	106
Mean % Recovery		**109.8**

DxC800-4449

Expected	Measured	Recovery (%)
0.00	0.00	100
0.50	0.60	120
1.00	1.15	115
2.00	2.30	115
4.00	4.30	108
8.00	8.60	108
Mean % Recovery		**111**

Expected	Measured	Recovery (%)
0.00	0.00	100
0.50	0.57	114
1.00	1.14	114
2.00	2.28	114
4.00	4.25	106
6.67	6.91	104
7.43	7.68	103
Mean % Recovery		**107.9**

Additional Information

For more detailed information on UniCel DxC Systems, refer to the *Instructions for Use* and *Reference* manual.

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