| ß **Hydroxybutyrate** |
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| **Purpose** | This procedure provides instructions for performing ß -HYDROXYBUTYRATE PROCEDURE on serum or plasma in Children’s Laboratory. |
| **Policy Statements** | * This procedure applies to all personnel who operate the Abbott Architect c4000.
* All components of individual ß-hydroxybutyrate reagent kits must be used together, and not shared between kits.
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| **Principle** | ß -Hydroxybutyrate (D-3-hydroxybutyrate) in the presence of NAD is 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. The NADH produced is converted to color using INT in the presence diaphorase to produce color at 505 nm.  |
| **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 ß-hydroxybutyrate.Ordinarily, ß-hydroxybutyrate is the ketoacid present in the greatest amount in serum. It accounts for approximately 75% of the ketone bodies that also contain acetoacetate and acetone. 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.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 shown by the blood levels of ß-hydroxybutyrateIn pediatric patients, the presence or absence of ketonemia/uria is an essential component in the differential diagnosis of inborn errors of metabolism |
| **Analyzer** | Abbott Architect c4000 (Sunquest method code: ARCH4) |
| **Sunquest Test Codes** | **BHBB** B Hydroxybutyrate |
| **Specimen** | Plasma (lithium heparin) preferred, or Serum (40 μL). Refer to specimen collection procedures for collection of diagnostic blood specimens by venipuncture.**Patient** **Preparation:** The reference range is for patients fasting for 9 – 12 hours prior to collection of the specimen.**Sample volume:** 0.2 mL preferred, 150 uL minimum.**Stability:** RT / 24 hours, 2-8 °C / 7 days, -20 °C / 1 month.**Rejection criteria**: Unlabeled specimens, or specimens other than heparinized plasma or serum |
|  | **Preparation:** 1. Complete clot formation should take place before centrifugation.
2. Serum or plasma should be physically separated from cells as soon as possible with a maximum limit of **two hours** from the time of collection.
3. Specimens should be free of particulate matter.
4. Whole blood specimens should be centrifuged according to Specimen Processing procedures prior to analysis. See Processing Procedure Manual.
5. Transfer serum, plasma, or body fluid to a properly labeled Siemens SSC nested on a bar-coded pilot tube. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.
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| **Materials** | **Supplies** | **Reagents** |
| * Automatic Pipettes and tips
* Sample Cups
* 55mL Abbott Architect Reagent Wedge
* 20 mL Abbott Architect Reagent Wedge
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| Unopened Stability  | Unopened Stability | Onboard/Open Stability |
| Stanbio Reagent A Enzyme (**R1**) 50 mL (β-hydroxybutyrate dehydrogenase and diaphorase enzymes)  | Until printed expiration date at 2 - 8°C  | For 30 days at 2 – 8°C  |
| Stanbio Reagent B Catalyst (**R2**) 8.5 mL (NAD, INT, and oxalate). Protect from light. | Until printed expiration date at 2 - 8°C  | For 30 days at 2 – 8°C  |
| Stanbio Standard/Calibrator: 1.00 mmol/L of β-Hydroxybutyrate | Until printed expiration date at 2 - 8°C | 60 Days |

Do not mix reagents for β-hydroxybutyrate between different kits. Use all reagents within the same kit lot together, or discard. |
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| **Risk and Safety** | *For In Vitro Diagnostic Use Only.* The reagents contain Sodium Azide (0.095 %) as preservative.Do not swallow! Avoid contact with skin and mucous membranes! If this occurs wash immediately with water. |
|  | **STEP**  | **ACTION**  |
| **Preparing and Loading Reagent** |  | Each Stanbio reagent kit contains reagent for 2 kits. Use half (25 mL) of the R1 and half (4.25 mL) of the R2 reagents per kit. Label the box that the kit has been opened using date and initials to signify there is only one kit left in the box. |
|  |  | Obtain a 55 mL wedge and a 20 mL wedge for the Abbott Architect c4000. Label each with the Manufacturer’s printed lot number, onboard stability expiration date (30 days), and the Serial Number entered according to the following scheme:Serial number is equal to the current date in the four-digit format, MMDD. For example, the serial number for October 7 is 1007.  |
|  |  | Label the 55 mL wedge as R1 and the 20 mL wedge as R1. |
|  |  | Fill the R1 55 mL wedge with 25 mL of R1 reagent, and the R2 20 mL wedge with 4.25 mL. Ensure there are no bubbles or droplets on the bottle opening or on the surface of the reagent as this will cause erroneous assay results. Use a pipette to remove them, if necessary. |
|  |  | Configure the reagent using the procedure outlined in the Abbott Architect c4000 Operating Procedure section **Configuration of Non-barcoded Reagents and Diluents**. |
|  |  | Assign the R1 and R2 reagent locations using the CH5.107 Abbott Architect c4000 Operating Procedure section **Loading Non-barcoded Reagent**. |
|  |  | Place the wedges in the assigned locations as determined in step 6. |
|  |  | Scan reagents, or simply place the analyzer back into Running status. |
| **Calibration** |

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| Assay Range: | 0.02 – 4.50 mmol/L |
| Reference Material: | Stanbio Laboratory β-hydroxybutyrate LiquiColor® Calibration Standard (1.0 mmol/L) Sodium D-3-hydroxybutyrate) |
| Suggested Calibration Levels: | 0.0, 1.00 |
| Calibration Scheme: | Two levels in triplicate |
| Calibration Frequency: | * For each new lot of Stanbio β-hydroxybutyrate LiquiColor® Reagent
* Every 72 Hours
* After major maintenance or service, if indicated by quality control results
* As indicated in laboratory quality control procedures
* When required by government regulations
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| Assigned Coefficients: | C0 0.000 C1 1.000 |
| Analytical Measuring Range | 0.02 – 4.50 mmol/LVerify the AMR once every 6 months with Audit Microcontrols Product #K728M-5, Linearity LQ Beta-Hydroxybutyric Acid.* Run all 5 standards **in triplicate** as patient samples. Assay results are submitted to Audit Microcontrols for compilation and comparison to peers. Results are reviewed and approved by the Technical Specialist. Any questionable results are investigated and corrective actions documented.
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| **Quality Control** | Stanbio Laboratory TDM/β-Hydroxybutyrate Tri-Level Controls(Cat. 2460-605)**Frequency**: * Two levels of controls must be run every 24 hours
* When loading a new set of reagent cartridges
* After calibration
* After any major maintenance/ repairs have been performed on the analyzer
* When questionable results are obtained

**Storage and Stability:** **Unopened:** 2°- 8°C until expiration date on label.**Open:** stable for 60 days at 2°- 8°CDiscard if turbidity or any change in appearance occurs.**Procedure**Gently mix the control solution by inversion. The control is ready for use and should be treated in the same manner as a patient test sample. Replace plug and cap and store at 2°- 8°C. Use only as directed.**Unity Real Time Control names:** Level 1 = BHOBLOWXXXXXXLevel 3 = BHOBHIGHXXXXXXWhere XXXXXX represents the manufacturer’s master lot number.**NOTE**: High QC is “Level 2” in Unity Real Time. The medium QC level is not utilized.**Acceptable ranges:** * Non-Bio-Rad controls will utilize manufacturer ranges and 2 SD Westgard rules.
* New lots of Bio-Rad controls should be run for 20 days in parallel with the current lot whenever possible prior to switching to the new lot.
* Refer to the Westgard Rules in Chemistry procedure (insert hyperlink) for current Westgard rules in place for each analyte.
* **Acceptable ranges are current in Unity Real Time only.** Quality Control results must be rejected in Sunquest when the results cross the interface.
* In the event of a QC failure, refer to the [Unity Real Time QC Review, General User](https://starnet.childrenshc.org/References/labsop/chem/quality/ch-2.17-unity-real-time-qc-review-general-user.pdf) and navigate to the QC Troubleshooting section.
* Do not load or release patients until QC is acceptable in Unity Real Time.
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| **Interferences** | * 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.
* Moderate hemolysis demonstrated no interference.
* See the product insert for a list of substances for which no significant interference was noted.
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| **Reference Range** | Fasting: **0.02 – 0.27 mmol/L**Non-fasting (random): <0.4 mmol/L 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 to 0.27 mmol/L. |
| **Critical Values** | None defined. |
| **Limitations** | Linear range of detection: **0.02 – 4.5** mmol/LThe instrument reporting system contains flags and comments to provide the user with information regarding instrument processing errors, instrument status information and potential errors in open channel method results. Refer to the Abbott Architect Operators Guide for the meaning of report flags and comments. Any report containing flags and/or comments must be resolved prior to reporting. |
| **Dilutions** |

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| **Architect c4000:**  |
| Automated Instrument Dilutions: | 1:3, 1:10 |
| Maximum Automated Dilution: | 1:10 |
| Diluent: | Onboard Saline |
| Manual Dilution: | Follow Abbott [Architect Operator’s Manual](https://starnet.childrenshc.org/References/labsop/chem/operator/abbott-architect-operations-manual.pdf) instructions for programming automated dilutions. The system will use the dilution factor to automatically calculate the concentration of the sample and report the result. If a diluted sample result is less than the lower value of the measuring interval of 0.02 mg/dL, do not report the result. Rerun using an appropriate (lower) dilution or investigate for other possible causes. |

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| **Result Reporting** | * Results between 0.02 – 4.50 mmol/L without error messages are released
* Results below 0.02 mmol/L: report as < 0.02 mmol/L instead of the numerical value.
* Results >4.50 mmol/L should have the 1:3 automated instrument dilution performed.
* Results > 13.50 mmol/L following the automated instrument dilution should have the 1:10 automated instrument dilution performed.
* Results that exceed the assay range following the maximum automated dilution of 1:10 are reported as >45.00 mmol/L.
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| **Specimen Storage** | Promptly stopper tested specimen and store upright in specimen rack. Every 8 hours remove specimens to refrigerator/freezer storage. Samples are retained 7 days in specimen storage freezer. |
| **References** | Stanbio Laboratory B-hydroxybutyrate LiquiColor Procedure # 2440, 1261 North Main Street, Boerne, TX 78006, 4/20171. Architect System Assay Parameters, ß-Hydroxybutyrate LiquiColor®, Stanbio Chemistry from EKF Diagnostics, Boerne TX, Effective Date 11/15/2018.
2. Abbott Architect Operations Manual, Abbott Diagnostics Division, Abbott Park, IL. 04/29/2016
3. Stanbio Laboratory Tri-Level TDM/Β-hydroxybutyrate Controls, Product Insert, Ref # 2460, 6/2015
4. Sena, Salvador F. *Technically Speaking*, Danbury Hospital Department of Pathology and Laboratory Medicine, Vol 4, No. 8, 07/2010
5. Mayo Medical Laboratories, online test catalogue, 01/2012
6. Jacobs & DeMott Laboratory Test Handbook, Lexi-Comp, Inc., Hudson, OH, 5th Edition, 2001, p.205.
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | Linda Lichty | 2/7/2012 | New test |
|  | Linda Lichty | 6/28/13 | Added policy, do not mix reagents between kits |
|  | Linda Lichty | 6/1/15 | Omit retired RxL as method |
|  | Linda Lichty | 9/1/2016 | Added instructions to assure QC is performed on newly loaded flex. |
|  | Erin Bartos | 10/15/2019 | Updated procedure for Abbott Architect c4000, new instrumentation. |