PRINCIPLE

Ketosis is a common feature in acutely ill patients. In patients 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 B-hydroxybutyrate, which accounts for 75% of ketone bodies. Other ketone bodies are acetoacetate and acetone. This test is appropriate in response to orders for “ketones” or ketone bodies”. Orders for “acetone” require clarification with the ordering physician to determine if the intent is to diagnose or monitor ketoacidosis or to detect environmental exposure. The latter requires send out testing to a reference lab that utilitizes Gas Chromatography, Mass Spectrometry, or similar methods for acetone detection.

PROCEDURE

The test is run on the C501 Roche Cobas. NAD is reduced to NADH in the presence of B-hyroxybutryate at an alkaline pH. NADH subsequently reacts with INT in the presence of diaphorase to produce color at 505 nm. Spectrophotometric measurement of the color produced is proportional to the concentration of B-hydroxybutyrate in the sample.

SPECIMEN

Serum or plasma collected with EDTA, heparin, or sodium fluoride can be used in the assay. Serum or plasma B-hyroxybutyrate levels are stable at least one week if kept refrigerated (2-8 C). Hemolyzed serum with an optical density at 540nm of 2.0 was added to the test and found not to interfere.

REAGENT

Stanbio Reagent A B-hydroxybutyrate Enzyme (R1) 50mL

Stanbio Reagent B B-hydroxybutyrate Catalyst (R2) 8.5mL

Stanbio B-hydroxybutyrate Standard 3mL

Using a multi-use reagent cassette fill the R1(A) port with 24mLs Reagent A Enzyme. The maximum amount that can be loaded into the R1 port is 25mLs. Recap R1. There will be residual reagent A.

Pour all 8.5mLs of Reagent B, catalyst, into the R2(B) port, recap.

To load reagent on the Cobas select the **REAGENT** tab, select **SETTING**, select **OPEN CHANNEL,** select **BHU SB,** select **RESERVE,** load cassette into reagent loading slot.

CALIBRATION:

Using calibration rack 4, position 2, place approximately 0.5cc’s of Stanbio B-hydroxybutyrate Standard into a micro sample cup. Standard comes in a 3mL bottle with reagent kit. Select 2 point calibration. Calibrate with every new cassette, when troubleshooting QC issues and whenever there are problems with the assay.

If calibration fails, repeat. If calibration fails a second time use fresh calibrator. If unresolved prepare a new cassette, if available. If calibration is still unacceptable call Hotline 1-800-531-5535. Do not report any patient values until all issues are resolved and QC is acceptable.

QUALITY CONTROL

Two levels of control material (low and high) will be analyzed on the instrument every 24 hours. We will be using Stanbio TDM/B-Hydroxybutyrate Tri-Level Controls 2 x 5mL. Once open control is good 60 days refrigerated (2-8C).

REPORTING RESULTS

The procedure is linear to 8.0mM B-hydroxybutyrate. Report out as >8.0 mmol/L. Do not dilute.

EXPECTED VALUES

The quantization of B-hydroxybutyrate is important in cases of ketoacidosis. In studies of healthy individuals who had fasted for 12 hours before blood collection the range was found to be 0.02 – 0.27 mmol/L. Suggested interpretive ranges according to the manufacturer are: Diabetic ketoacidosis resolved <0.5 mmol/l, Diabetic ketoacidosis not resolved >1.1 mmol/L.

LIMITATIONS

Lactic dehydrogenase and lactate have been shown to interfere with the assay, but the incorporation of oxalic acid in this reagent eliminates this interference as reported.

ATTACHMENTS N/A

REFERENCES Package Insert Procedure, Stanbio Laboratory, 1261 North Main Street, Boerne, TX 78006, Procedure No. 2440CE: RBR.2440.CE.01 Revision: 06/15