Ortho Clinical Diagnostics

Performance of the EKF Diagnostics, Stanbio β-Hydroxybutyrate LiquiColor® Assay on the VITROS® 4600 Chemistry System and the VITROS® 5600 Integrated System.

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Introduction

- The EKF Diagnostics, Stanbio β-Hydroxybutyrate LiquiColor assay quantitatively determines the concentration of β-Hydroxybutyrate (BHB) in serum or plasma.
- β-Hydroxybutyrate, acetoacetate and acetone are three ketogenic byproducts from the metabolism of fatty acids. The byproducts are typically present in low concentration; however in disease state conditions such as diabetic ketoacidosis the levels are elevated, with β-Hydroxybutyrate being present in the highest concentration.
- The traditional method for detecting the ketogenic byproducts is the qualitative nitroprusside test which detects only the ketone bodies acetoacetate and acetone, but fails to detect the ketoacid, β-Hydroxybutyrate, the most prominent “ketone body”.
- Quantitatively measuring β-Hydroxybutyrate provides a more accurate method for diagnosis and monitoring of diabetic ketoacidosis.

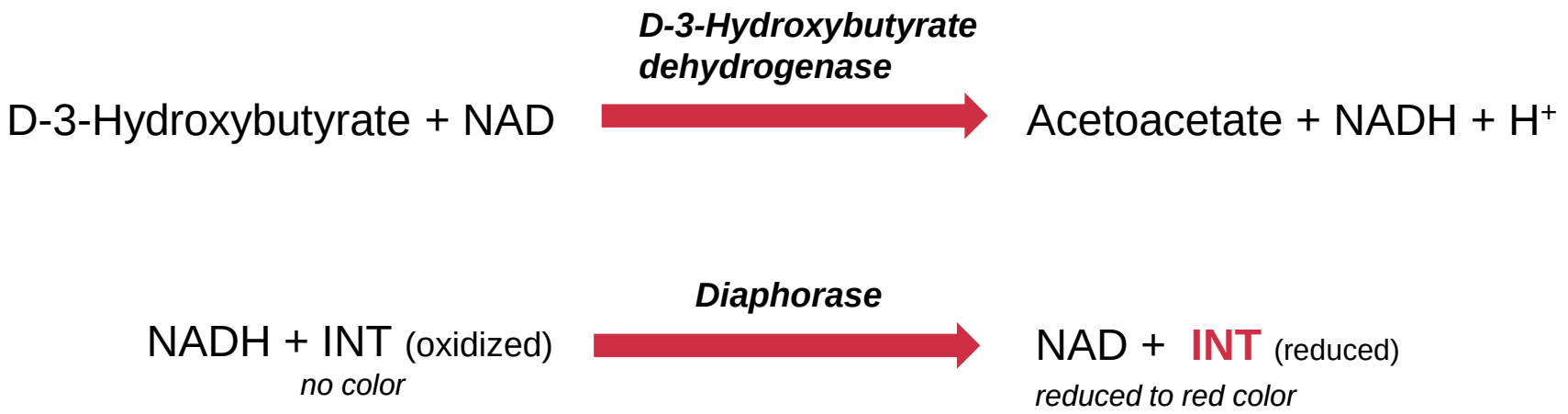
Sources:

1. Sacks, DB et al. *Guidelines and Recommendations for Laboratory Analysis and in the Diagnosis and Management of Diabetes Mellitus.* Diabetes Care, 2011; 34:e61–e99.
2. Shiekh-Ali M, et al. *Can Serum beta-Hydroxybutyrate be used to diagnose diabetic ketoacidosis?* Diabetes Care, 2008; 31:643-647.

Assay Method Overview

- The performance of the Stanbio β-Hydroxybutyrate LiquiColor assay on the VITROS 4600 Chemistry System (VITROS 4600) and the VITROS 5600 Integrated System (VITROS 5600) was evaluated.
- The assay was run on the VITROS MicroTip assay processing side of the MicroImmunoassay Center using 4.0uL of sample and the two Stanbio LiquiColor reagents.
- Endpoint absorbance measurements were taken at 510nm and converted to a concentration using a linear calibration model. The absorbance is directly proportional to the concentration of β-Hydroxybutyrate in the sample.

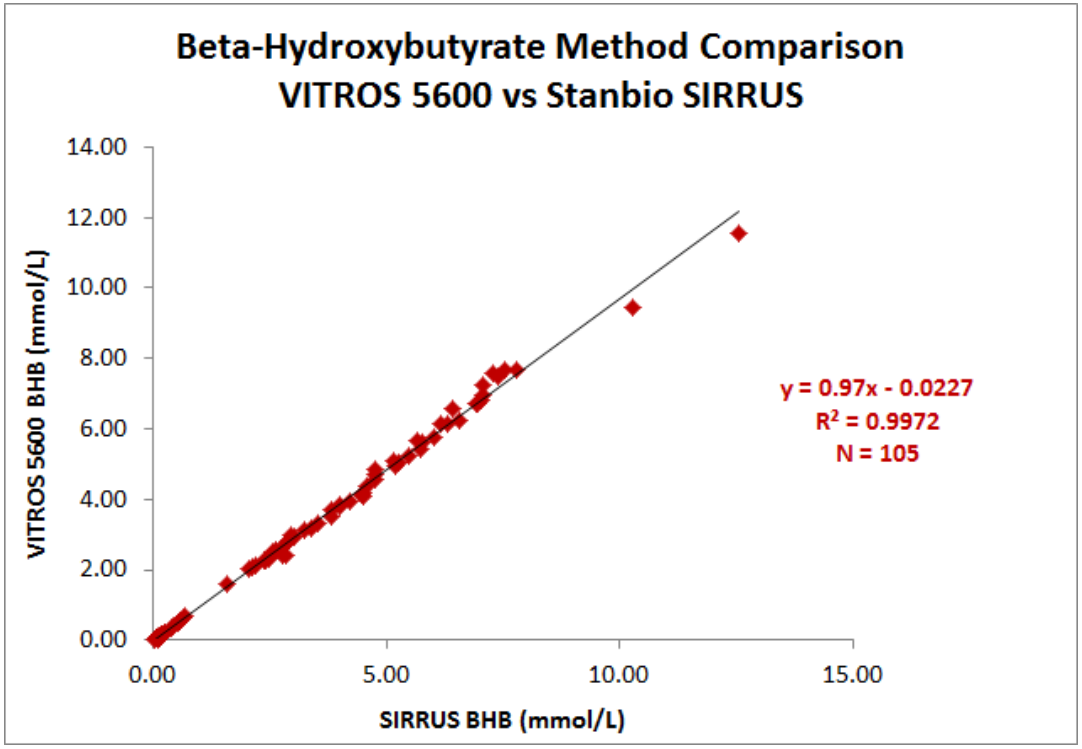
Assay Enzymatic Reaction Scheme



Patient sample β-Hydroxybutyrate in the presence of NAD is converted to acetoacetate and NADH by β-Hydroxybutyrate dehydrogenase. NADH produced by this reaction reacts with 2-(4-iodophenyl)-3(4-nitrophenyl)-5-phenyl-2H-tetrazolium dye (INT) in the presence of diaphorase to generate a colorimetric signal at 510nm.

Method Comparison

- A split sample comparison was conducted with 105 serum and plasma samples (0.05 – 12.56 mmol/L β-Hydroxybutyrate) using the VITROS 4600/5600 Systems and the Stanbio SIRRUS Clinical Chemistry Analyzer.
- The VITROS 4600 and VITROS 5600 Systems showed excellent correlation with the SIRRUS Analyzer.



Data shown from VITROS 5600 Integrated System

Precision

- Total within-laboratory precision was evaluated in accordance with CLSI EP05-A3 by evaluating β-Hydroxybutyrate controls at 0.191 mmol/L and 4.224 mmol/L in duplicate twice per day for 28 days, for a total of 112 replicates.
- Precision components generated using Minitab® software are shown for the VITROS 4600 and VITROS 5600 Systems.

ANOVA Components	VITROS 4600		VITROS 5600	
	Control 1	Control 2	Control 1	Control 2
Mean	0.194	4.214	0.188	4.234
Between-Day SD	0.002	0.017	0.001	0.016
Between-Run SD	0.001	0.017	0.001	0.023
Within-Run SD (Repeatability)	0.005	0.028	0.002	0.025
Total SD (Within-Lab)	0.005	0.037	0.002	0.037
Total %CV	2.58%	0.88%	1.06%	0.87%
Within-Run %CV	2.58%	0.66%	1.06%	0.59%

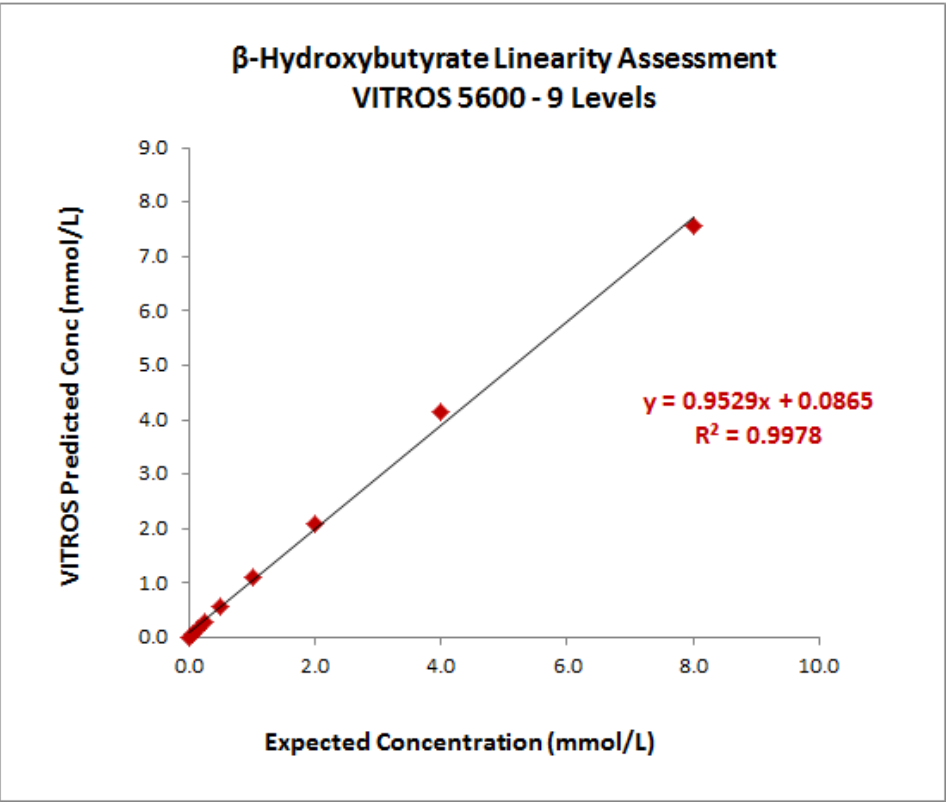
Limit of Quantitation

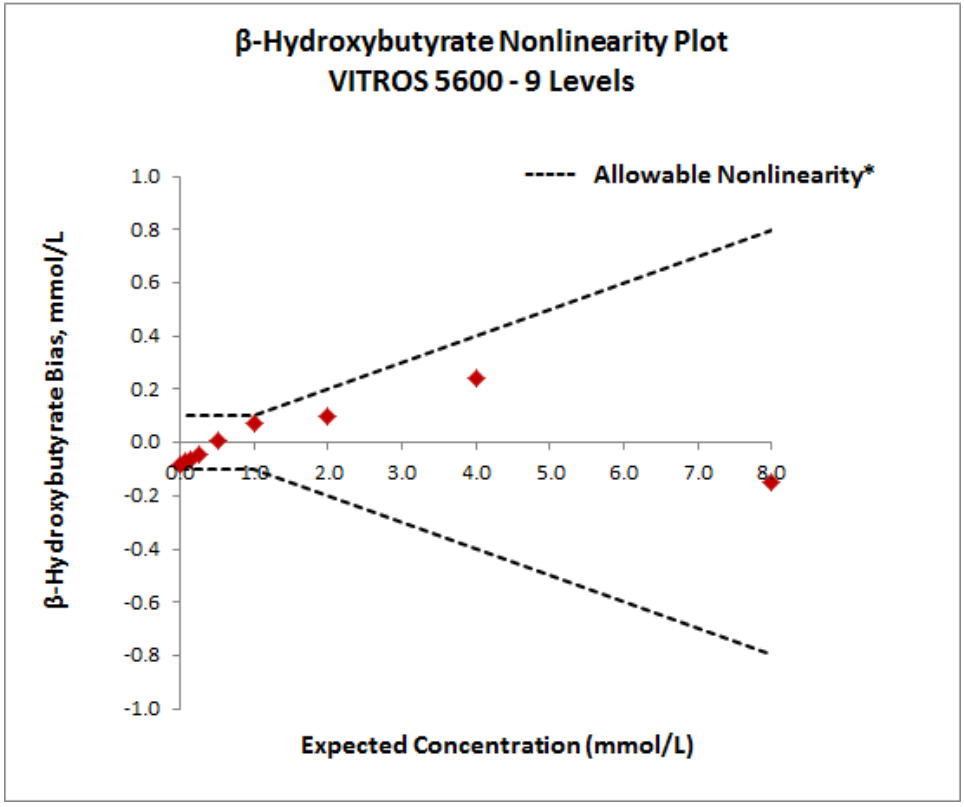
- The Limits of Blank (LoB), Detection (LoD) and Quantitation (LoQ) were determined in accordance with CLSI EP17-A2.
- LoB and LoD were determined by the non-parametric and parametric methods, respectively as described in the CLSI guideline.
- LoQ was determined by the precision profile approach as the predicted concentration at which the precision estimate was ≤ 10% CV.
- The values reported are the most conservative across the VITROS 4600 and VITROS 5600 Systems.

VITROS System	LoB (mmol/L)	LoD (mmol/L)	LoQ (mmol/L)
4600 / 5600	0.0102	0.0164	0.0315

Linearity

- The linearity of the Stanbio β-Hydroxybutyrate LiquiColor assay on the VITROS 4600 Chemistry System and the VITROS 5600 Integrated System was evaluated in accordance with CLSI EP06-A.
- The assessment was conducted using the Stanbio Laboratory TDM/beta-Hydroxybutyrate Linearity Standards. The observed linear range was 0.0 – 8.0 mmol/L BHB for both VITROS Systems.



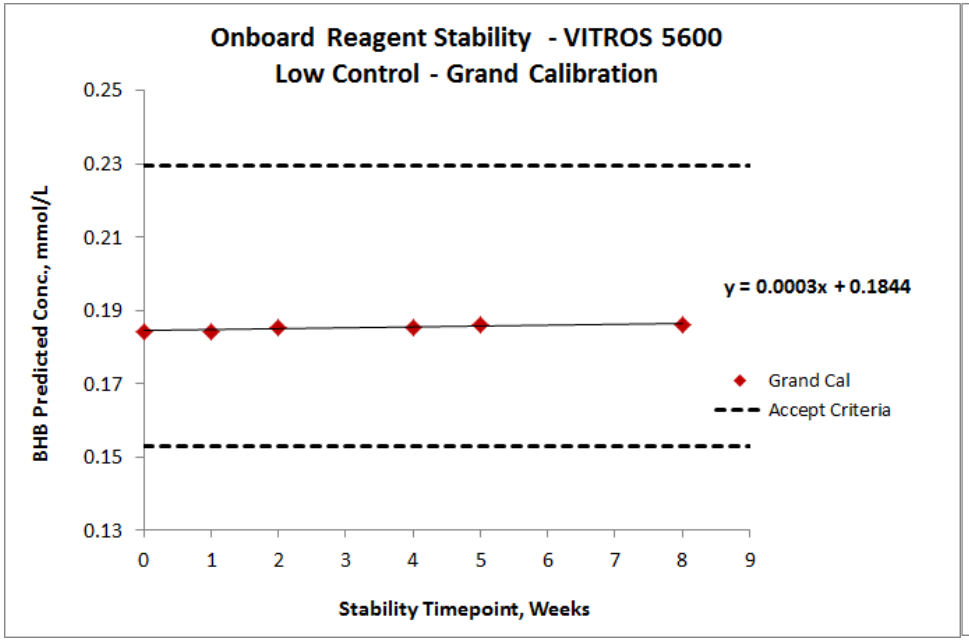


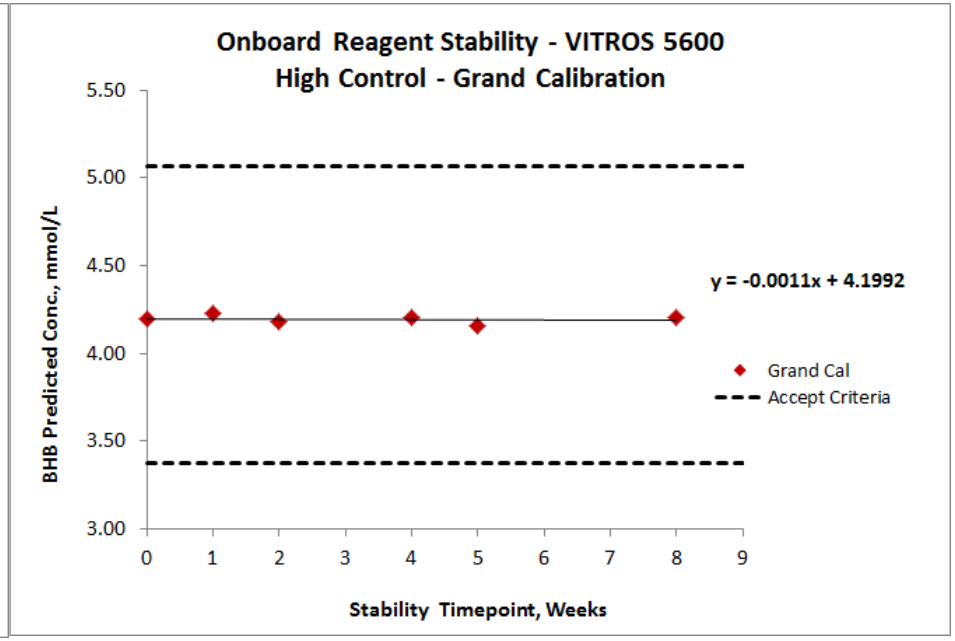
Data shown from VITROS 5600 Integrated System

***Allowable bias = 0.1 mmol/L at [BHB] ≤ 1.0mmol/L, <10% at [BHB] > 1.0mmol/L**

Onboard Reagent Stability

- The stability of the Stanbio Laboratory β-Hydroxybutyrate Liquicolor reagents while stored onboard the VITROS 4600 Chemistry System and the VITROS 5600 Integrated System was evaluated.
- Stanbio BHB Bi-level Controls were used as the test fluids with 4 replicates per timepoint. Mean values at each timepoint were plotted.
- Results indicate that the BHB reagent onboard stability is at least 8 weeks (56 days).

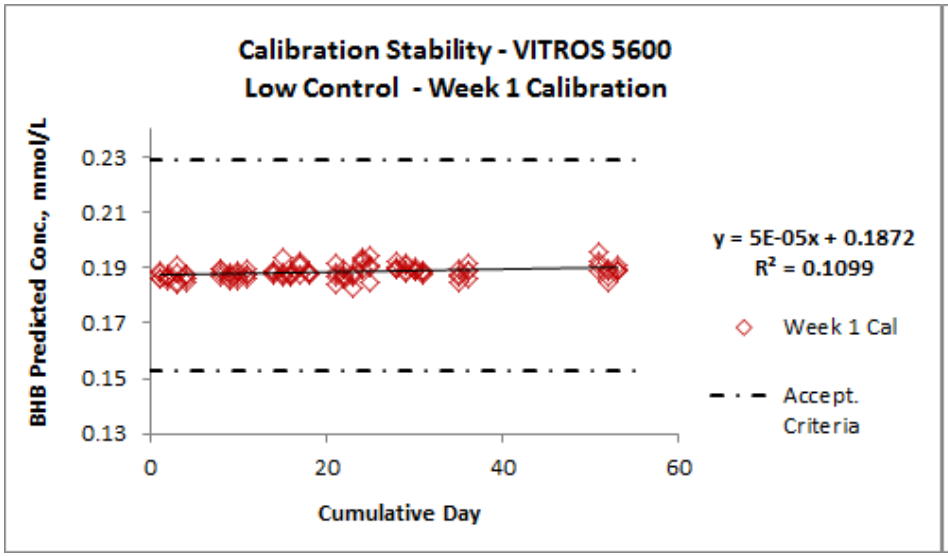


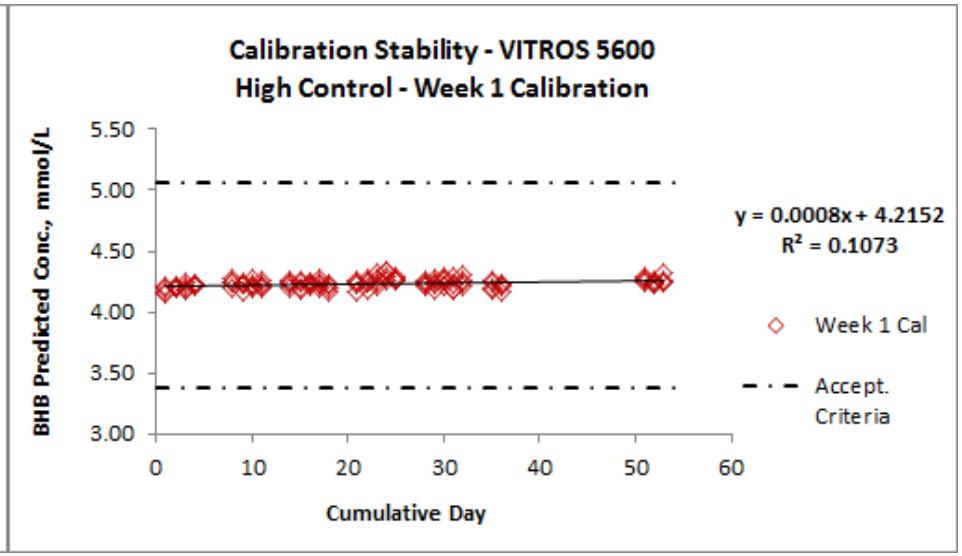


Data shown from VITROS 5600 Integrated System

Calibration Stability

- The calibration stability of the Stanbio β-Hydroxybutyrate LiquiColor assay on the VITROS 4600 Chemistry System and the VITROS 5600 Integrated System was evaluated.
- Stanbio BHB Bi-level Controls were used as the test fluids over 28 test days (53 cumulative days). Total replicates = 112.
- Results indicate that the BHB assay calibration stability is at least 53 days.





Data shown from VITROS 5600 Integrated System

Specificity

- Using the paired difference method from CLSI guideline EP07-A2 the performance of the Stanbio β-Hydroxybutyrate LiquiColor assay on the VITROS 4600 Chemistry System and the VITROS 5600 Integrated System was assessed in the presence of common endogenous substances.
- Endogenous substances at various concentrations were spiked into a 1.5 mmol/L β-Hydroxybutyrate serum patient sample pool and evaluated for assay interference. The values reported are the highest concentration of each endogenous substance which met the assay acceptance criteria for interference.

Endogenous Substance	VITROS 4600	VITROS 5600
Hemoglobin, mg/dL	600	600
Bilirubin - Unconjugated, mg/dL	40	40
Bilirubin - Conjugated, mg/dL	40	40
Triglycerides (Intralipid®), mg/dL	1600	1600

Conclusions

The performance of the EKF Diagnostics, Stanbio β-Hydroxybutyrate LiquiColor assay on the VITROS 4600 Chemistry System and the VITROS 5600 Integrated System was evaluated. The assay exhibited the following:

- ✓ Excellent correlation with the SIRRUS Clinical Chemistry Analyzer
- ✓ Excellent Within-Lab precision and low end sensitivity
- ✓ Onboard reagent stability to at least 56 days
- ✓ Calibration stability to at least 53 days
- ✓ The assay was free from interference by endogenous substances at a clinically relevant β-Hydroxybutyrate concentration.