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:
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.
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.
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.

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

<table>
<thead>
<tr>
<th>VITROS System</th>
<th>LoB (mmol/L)</th>
<th>LoD (mmol/L)</th>
<th>LoQ (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4600 / 5600</td>
<td>0.0102</td>
<td>0.0164</td>
<td>0.0315</td>
</tr>
</tbody>
</table>
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.

\[ y = 0.9529x + 0.0865 \]
\[ R^2 = 0.9978 \]

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

Data shown from VITROS 5600 Integrated System
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.

<table>
<thead>
<tr>
<th>Endogenous Substance</th>
<th>VITROS 4600</th>
<th>VITROS 5600</th>
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</thead>
<tbody>
<tr>
<td>Hemoglobin, mg/dL</td>
<td>600</td>
<td>600</td>
</tr>
<tr>
<td>Bilirubin - Unconjugated, mg/dL</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Bilirubin - Conjugated, mg/dL</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Triglycerides (Intralipid®), mg/dL</td>
<td>1600</td>
<td>1600</td>
</tr>
</tbody>
</table>
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.