3. Sensitivity
The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 20 times in the same run.

<table>
<thead>
<tr>
<th>Serum</th>
<th>No. of Replicates</th>
<th>Mean ng/ml</th>
<th>Standard Deviation</th>
<th>Mean + 2SD (Sensitivity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero Standard</td>
<td>20</td>
<td>0.23</td>
<td>0.14</td>
<td>0.51 ng/ml</td>
</tr>
</tbody>
</table>

REFERENCES

2010-04-21

Warning
All of BQ Kits ELISA kits have not been tested for clinical use and are not approved in the United States by the FDA for diagnostic clinical use. They are components or reagents made solely for research use, further manufacturing and export use. It is the commitment of BQ Kits customers to receive its products solely for the purpose of exportation or research, and not for the purposes of clinical diagnostic use.

BQ KITS, INC. DOES NOT MAKE ANY OTHER WARRANTY OR REPRESENTATION WHATSOEVER, WHETHER EXPRESS OR IMPLIED, WITH RESPECT TO THESE PRODUCTS. IN PARTICULAR BQ KITS, INC. DOES NOT MAKE ANY WARRANTY OF SUITABILITY, NON-INFRINGEMENT, MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE OF ANY PRODUCT.
STORAGE AND STABILITY
1. Store the kit at 2 - 8°C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose reagent to heat, sun, or strong light.

WARNINGS AND PRECAUTIONS
1. Potential biohazardous materials: The standard set contains human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, “Biosafety in Microbiological and Biomedical Laboratories.” 1984
2. This test kit is designed for research use only.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that standards, control and serum samples be run in duplicate.
6. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION HANDLING
1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20°C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

REAGENTS PREPARATION
Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26°C).

ASSAY PROCEDURE
Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use.
1. Place the desired number of coated strips into the holder.
2. Pipette 25µl of Ferritin standards, control and patient’s sera.
3. Add 100µl of incubation buffer to all wells. Shake the plate gently (20 seconds) to mix the reagents.
4. Cover the plate and incubate for 30 minutes at room temperature (18-26°C).
5. Remove liquid from all wells. Wash wells three times with 300µl of 1X wash buffer. Blot on absorbent paper towels.
6. Add 100µl of enzyme conjugate to all wells.
7. Cover the plate and incubate for 30 minutes at room temperature (18-26°C).
8. Remove liquid from all wells. Wash wells three times with 300µl of 1X wash buffer. Blot on absorbent paper towels.
9. Add 100µl of TMB substrate to all wells.
10. Incubate for 15 minutes at room temperature (18-26°C).
11. Add 50µl of stop solution to all wells. Shake the plate gently to mix the solution.
12. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

CALCULATION OF RESULTS
The standard curve is constructed as follows:
1. Check Ferritin standard value on each vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.
2. To construct the standard curve, plot the absorbance for the Ferritin standards (vertical axis) versus the Ferritin standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Example of a Standard Data

<table>
<thead>
<tr>
<th>Std</th>
<th>OD 450 nm</th>
<th>Conc. ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.04</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.23</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>0.79</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>1.54</td>
<td>150</td>
</tr>
<tr>
<td>5</td>
<td>2.24</td>
<td>400</td>
</tr>
<tr>
<td>6</td>
<td>2.62</td>
<td>800</td>
</tr>
</tbody>
</table>

EXPECTED VALUES
It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values may be used as initial guideline ranges only:
Men: 15-250 ng/ml
Women: 10-125 ng/ml

LIMITATIONS OF THE TEST
1. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

PERFORMANCE CHARACTERISTICS
1. Correlation with a Reference ELISA kit:
A total of 60 sera were tested by this kit and a commercially available ferritin reference ELISA kit. The linear regression curve was calculated as:
\[ Y = 1.1x + 0.168, \ r = 0.99 \]

2. Precision

<table>
<thead>
<tr>
<th>Serum</th>
<th>No. of Replicates</th>
<th>Mean ng/ml</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>31.7</td>
<td>1.45</td>
<td>4.6</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>90.3</td>
<td>2.16</td>
<td>2.4</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>266.1</td>
<td>10.5</td>
<td>3.9</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Serum</th>
<th>No. of Replicates</th>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>31.2</td>
<td>1.9</td>
<td>6.1</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>89.8</td>
<td>2.8</td>
<td>3.1</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>266.1</td>
<td>10.5</td>
<td>3.9</td>
</tr>
</tbody>
</table>