**PERFORMANCE CHARACTERISTICS**

1. **Sensitivity and Specificity**

   178 patient sera were tested by this Brucella IgM ELISA and a reference ELISA method. 26 sera were positive and 149 were negative by both methods (98% agreement). The results are summarized below:

<table>
<thead>
<tr>
<th>Brucella IgM ELISA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>23</td>
</tr>
<tr>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Reference ELISA Kit</td>
<td>1</td>
</tr>
<tr>
<td>+</td>
<td>152</td>
</tr>
<tr>
<td>-</td>
<td>153</td>
</tr>
<tr>
<td>Total</td>
<td>178</td>
</tr>
</tbody>
</table>

2. **Precision**

   **Intra Assay Study**

<table>
<thead>
<tr>
<th>Serum</th>
<th>No. of Replicates</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>1.49</td>
<td>0.066</td>
<td>4.43</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>1.01</td>
<td>0.051</td>
<td>5.50</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>0.19</td>
<td>0.012</td>
<td>6.31</td>
</tr>
</tbody>
</table>

   **Inter Assay Study**

<table>
<thead>
<tr>
<th>Serum</th>
<th>No. of Replicates</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>1.41</td>
<td>0.139</td>
<td>9.65</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>0.97</td>
<td>0.100</td>
<td>10.30</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>0.20</td>
<td>0.022</td>
<td>11.00</td>
</tr>
</tbody>
</table>

**REFERENCES**

3. Bowden RA; Cloeckaert A; Zygmunt MS; Bernard S; Dubray G. Surface exposure of outer membrane proteins and lipopolysaccharide epitopes in Brucella species studied by enzyme-linked immunosorbent assay and flow cytometry. Infect Immun 1995; 63(10):3945

**MATERIALS PROVIDED**

- **MATERIALS PROVIDED**

  1. Microwell coated with Brucella antigen: 12x8x1
  2. Sample Diluent: 1 bottle (ready to use): 22 ml
  3. Calibrator: 1 Vial (ready to use): 1.5 ml
  4. Positive Control: 1 vial (ready to use): 1.5 ml
  5. Negative Control: 1 vial (ready to use): 1.5 ml
  6. Enzyme conjugate: 1 bottle (ready to use): 12 ml
  7. TBS Substrate: 1 bottle (ready to use): 12 ml
  8. Stop Solution: 1 bottle (ready to use): 12 ml
  9. Wash concentrate 20X: 1 bottle: 25 ml

**MATERIALS NOT PROVIDED**

- **Distilled or deionized water**
- **Precision pipettes**
- **Disposable pipette tips**
- **ELISA reader capable of reading absorbance at 450nm**
- **Absorbance paper or paper towel**
- **Graph paper**
ASSAY PROCEDURE
1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 µl of the sample to 200 µl of sample diluent. Mix well.
3. Dispense 100 µl of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100 µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
7. Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100 µl of stop solution.
9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

CALCULATION OF RESULTS
1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
2. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

Example of typical results:
- Calibrator mean OD = 0.8
- Calibrator Factor (CF) = 0.5
- Cut-off Value = 0.8 x 0.5 = 0.400
- Positive control O.D. = 1.2
- Ab Index = 1.2 / 0.4 = 3
- Patient sample O.D. = 1.6
- Ab Index = 1.6 / 0.4 = 4.0

QUALITY CONTROL
The test run may be considered valid provided the following criteria are met:
1. The O.D. of the Calibrator should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab index for Positive control should be greater than 1.2.

INTERPRETATION
The following is intended as a guide to interpretation of Brucella IgM test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

Antibody Index Interpretation
- <0.9: No detectable antibody to Brucella IgM by ELISA.
- 0.9-1.1: Borderline positive. Follow-up testing is recommended if clinically indicated.
- >1.1: Detectable antibody to Brucella IgM by ELISA.

LIMITATIONS OF THE TEST
1. To enhance sensitivity and specificity of this IgM test provided sample diluent has been formulated to block IgG and Rheumatoid Factor (RF) interferences. Turbidity could be seen after diluting serum with sample diluent. This turbidity is due to the blocking of serum IgG and shows no interference with test results. It can be removed by centrifugation.
2. In specimens with high RF and high autoimmune antibodies, the possibility of eliminating the interferences cannot be ruled out entirely.
3. The test results obtained using this kit are for research use only and are not intended to be used as a part of any official diagnosis.
4. Lipemic or hemolyzed samples may cause erroneous results.