BRUCELLOSIS ANTIGEN FOR COMPLEMENT FIXATION TEST

The antigen for the Complement Fixation test is a concentrated suspension of Brucella abortus (strain S99 - Weybridge) inactivated by heat and phenol. This antigen allows the serological diagnosis of Brucellosis (Brucella melitensis, abortus, suis). This antigen is standardised to give 50% of fixation with a final dilution of 1/200 of the International Brucella abortus Standard Serum (OIEISS).

PRINCIPLE
The Complement Fixation test is one of the most sensitive Brucellosis diagnosis methods. A cold fixation method of the type developed by Kolmer is used.

REAGENTS
- Concentrated Brucellosis antigen to be diluted just before use in salt water (8.5 g/l) or better in veronal buffer.
- Veronal Buffer to be diluted to 1/5 in distilled water
- Freeze-dried Complement
- Sheep red blood cells at 50%
- Standardised haemolytic serum
- Sera to be tested
- Control sera

INSTRUCTIONS FOR USE

Titration of the complement
- Realise this titration in tubes
- Reconstitute the freeze-dried complement with the solvent as indicated. Dilute to 1/100 in Veronal buffer.
- Dilute the brucellosis antigen as recommended by the manufacturer
- Dispense in tubes according to the following table:

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>H0</th>
<th>H100</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. at 1/100 (ml)</td>
<td>0.04</td>
<td>0.05</td>
<td>0.06</td>
<td>0.07</td>
<td>0.08</td>
<td>0.09</td>
<td>0.10</td>
<td>0.11</td>
<td>0.12</td>
<td>0.13</td>
<td>0.14</td>
<td>0.15</td>
<td>0.16</td>
<td>0</td>
<td>0.40</td>
</tr>
<tr>
<td>Veronal Buffer</td>
<td>0.36</td>
<td>0.35</td>
<td>0.34</td>
<td>0.33</td>
<td>0.32</td>
<td>0.31</td>
<td>0.30</td>
<td>0.29</td>
<td>0.28</td>
<td>0.27</td>
<td>0.26</td>
<td>0.25</td>
<td>0.24</td>
<td>0.40</td>
<td>0</td>
</tr>
<tr>
<td>Ag diluted</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Shake the tubes - place them in a water-bath at 37°C for 30 minutes

Preparation of the haemolytical couple
The complement titration and Complement Fixation test must imperatively be realised with the same haemolytical couple elements. So, provide for sufficient quantities of products for these 2 uses. Store them separately between use.

- Mix in equivalent proportions:
  - The sheep red blood cell suspension at 2.5% (0.5 ml of sheep red blood cells at 50% and 9.5 ml of Veronal buffer).
  - The haemolytic serum diluted according to the titre indicated
Mix the quantities required for the complement fixation 20 min before use and leave at room temperature (between 18° and 23°C). Remaining products must be stored separately at +2°-+8°C until the following day.

Remove the tubes from the water-bath and centrifuge them immediately during 10 min to 500-1000 g

Prepare a H50 control : 0,50 ml of the H0 haemolytic control supernatant + 0,50 ml of the H100 haemolytic control supernatant. The first tube with a supernatant presenting the same colour than this H50 control will be called H50 unit

**Quantity of complement**

Example : Number of wells = 100

0,025 ml of diluted complement are dispensed in each well :

0,025 ml x 100 = 2,5 ml (total volume of diluted complement)

The H50 unit has been found for the tube n° 5 (0,08 ml of complement to 1/100).
The test uses 6 H50 units :

\[
0.08 \times 6 \times 100 \times 0.025 = 0.060 \text{ ml} \\
100 \times 0.2
\]

**Dilution of sera**

- Inactivate the sera to be titrated in a water-bath at 60°C for 30 min.
- Dilute them in Veronal buffer (in tubes or microplates)

**First solution : Dilution in tubes**

<table>
<thead>
<tr>
<th>Dilutions</th>
<th>1/2</th>
<th>1/4</th>
<th>1/8</th>
<th>1/16</th>
<th>1/32</th>
<th>1/64</th>
<th>1/128</th>
<th>1/256</th>
<th>1/512</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (ml)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Veronal Buffer (ml)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

**Second solution : Dilution in microplates**

25 µl eliminated

<table>
<thead>
<tr>
<th>Dilutions</th>
<th>T5</th>
<th>1/4</th>
<th>1/8</th>
<th>1/16</th>
<th>1/32</th>
<th>1/64</th>
<th>1/128</th>
<th>1/256</th>
<th>1/512</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veronal Buffer (µl)</td>
<td>75</td>
<td>/</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

**REACTION**

The reaction is implemented in plates

**For sera diluted in tubes**

<table>
<thead>
<tr>
<th>Serum dilution</th>
<th>1/2</th>
<th>1/4</th>
<th>1/8</th>
<th>1/16</th>
<th>1/32</th>
<th>1/64</th>
<th>1/128</th>
<th>1/256</th>
<th>1/512</th>
<th>TAg</th>
<th>TC'</th>
<th>TGR</th>
<th>T$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veronal Buffer (µl)</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>25</td>
<td>50</td>
<td>75</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

**For sera diluted in plates**

<table>
<thead>
<tr>
<th>Serum dilution</th>
<th>T$</th>
<th>1/4</th>
<th>1/8</th>
<th>1/16</th>
<th>1/32</th>
<th>1/64</th>
<th>1/128</th>
<th>1/256</th>
<th>1/512</th>
<th>TAg</th>
<th>TC'</th>
<th>TGR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veronal Buffer (µl)</td>
<td>25</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>/</td>
<td>25</td>
<td>50</td>
<td>75</td>
<td>25</td>
</tr>
</tbody>
</table>

- Shake plates on a plate agitator
- Cover the plates
- Incubate overnight at +2°C-+8°C
• Prepare the haemolysis couple mixing equivalent volumes of red blood cell suspension and haemolytic serum solution (prepared on previous day)
• Leave 10 minutes at room temperature (+18°C - +23°C)
• Then, place the plates in the incubator at 37°C for 10 minutes (either superposed with an aluminium foil between each plate, or side by side)
• The haemolysis couple has been kept 20 minutes at room temperature (+18°C - +23°C)
• Add 50µl of the haemolysis couple into each well.
• Shake the plates then cover them
• Place the plates in the incubator at 37°C for 30 minutes

**READING**

• Centrifuge the plates to 500-1000g, at +2°C - +8°C, for 10 minutes or leave them for 1 hour in the refrigerator at +2°C - +8°C
• Evaluate the supernatant according to the controls prepared as follows:

<table>
<thead>
<tr>
<th>Veronal Buffer (µl)</th>
<th>0</th>
<th>+</th>
<th>++</th>
<th>+++</th>
<th>++++</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total haemolysis well (µl) = Complement control</td>
<td>100</td>
<td>75</td>
<td>50</td>
<td>25</td>
<td>0</td>
</tr>
</tbody>
</table>

In each well, the sensibilised red blood cell haemolysis rate is noted as follows:

+++ = Total haemolysis inhibition
+++ = 75% of haemolysis inhibition (=25% of haemolysis)
++ = 50% of haemolysis inhibition (=50% of haemolysis)
+ = 25% of haemolysis inhibition (=75% of haemolysis)
0 = Complete haemolysis

**INTERPRETATION**

< 50% of haemolysis inhibition to 1/4 = **negative**
> 50% of haemolysis inhibition to 1/4 = **positive**

**Correspondence between the dilution of serum and the sensitizing EEC units (limit 50% of the haemolysis inhibition):**

<table>
<thead>
<tr>
<th>Serum Dilution</th>
<th>1/2</th>
<th>1/4</th>
<th>1/8</th>
<th>1/16</th>
<th>1/32</th>
</tr>
</thead>
<tbody>
<tr>
<td>EEC Units</td>
<td>10</td>
<td>20</td>
<td>40</td>
<td>80</td>
<td>160</td>
</tr>
</tbody>
</table>

**KIT CONTENTS**

1 x 100 ml vial of antigen for the reaction of the Complement Fixation

**STORAGE**

Store at +2°C - +8°C for 2 years, away from light. Do not freeze.

**PRODUCT CODES:**

- P00120 Brucellosis Antigen for Complement Fixation
- P00130 Positive Control
- P00121 Veronal Buffer (100 ml vial)

**IN VITRO USE**

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