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1. **PRINCIPLE**

The complement system consists of a complex series of proteins which, if triggered by an antigen-antibody complex react in a sequential manner to cause cell lysis. In the first stage of the CFT antigen and test serum are mixed with normal guinea pig serum (complement). In the second stage the indicator or haemolytic system is added. The haemolytic system consists of sheep red blood cells which have been sensitised with anti-sheep red blood cell antibody (haemolysin). If the test serum contains antibodies to *Brucella ovis* (*B. ovis*) (positive reaction) complement will be used up or fixed so that it cannot react in the haemolytic system. Thus no lysis of sheep red blood cells will occur and the sheep red blood cells will remain intact. If the test serum does not contain *B. ovis* antibodies (negative reaction) complement will not be fixed and lysis of the sheep red blood cells will occur.

2. **PERSONNEL**

The person/s performing the procedure shall be fully trained and certified competent, or should perform the procedure under supervision of a competent person.

3. **LIMITATIONS AND PRECISION OF THE TEST METHOD**

3.1 Complement factors and other factors inducing anti-complementary effects inherent in the test sera, have to be inactivated before performing the CFT.

3.2 The various biological reagents used in the CFT must all be standardized in order to yield optimal results.

4. **EQUIPMENT**

4.1 Multi-channel micropipette and single channel micropipette
4.2 Incubator
4.3 Timer
4.4 Refrigerator
4.5 Water bath
4.6 Centrifuges (1 Standard, 1 to spin microtitre plates)
4.7 Dispenser
4.8 Shaker
4.9 X-ray viewing box or magnifying mirror
4.10 Spectrophotometer.

5. **MATERIALS**

5.1 Koki pen
5.2 New test tubes
5.3 New pipette tips
5.4 Beaker
5.5 96-well microtitre plate filled with 1.2ml polypropylene cluster tubes (*Costar® 4408 supplied by Adcock Ingram – Scientific group*)
5.6 96-well U-bottom microtitre plates
5.7 Reagent bottles and reservoirs
5.8 Centrifuge tubes
5.9 Measuring cylinders.
6. **REAGENTS**

6.1 *Brucella ovis* antigen (Supplied by Bloemfontein Veterinary Laboratory)
6.2 Positive *Brucella ovis* sera (Supplied by Stellenbosch Veterinary Laboratory)
6.3 *Brucella ovis* negative sera (Supplied by Stellenbosch Veterinary Laboratory)
6.4 Guinea pig Complement for CFT- ORAY® (supplied by Dade Behring)
6.5 Amboceptor from rabbit- ORLC® (supplied by Dade Behring)
6.6 Veronal buffer - Virion® (supplied by Combined Medical Supplies)
6.7 3% Sheep red blood cell suspension
6.8 Test Sera

7. **SAFETY/ PRECAUTIONARY MEASURES**

7.1 Safety measures

7.1.1 Wear protective clothing and gloves at all times.
7.1.2 Spills must be cleaned by spraying with 70% alcohol and wiped down with paper towels. Paper towels must be discarded into a biohazard bag.

7.2 Precautionary measures

7.2.1 All reagents must be stored at 4°C at all times when not in use.
7.2.2 Antigen and complement to be mixed well before use.
7.2.3 Remove only a sufficient amount of reagents from refrigerator for use.

8. **PROCEDURE**

8.1 **Collection and preservation of sheep red blood cells (SRBC)**

8.1.1 Donor animals should be tested free from *Brucella ovis* antibodies.
8.1.2 Samples will be collected at a ratio 50% blood to 50% Alsever’s solution (Blood packs Optional – to be used as per manufacturer’s instructions).
8.1.3 Blood will be stored at 4°C (±2°C) for a period of no longer than 30 days.
8.1.4 Blood can only be used after 48 hours post collection.

8.2 **Preparation of reagents**

Reagents must reach room temperature (22°C ±3°C) before use, with the exception of the complement, which is heat labile and should be kept at 4°C until needed for dilution.

8.2.1 Preparation of Haemolytic System

A. **Washing SRBC**

1. Pour sufficient blood/Alsever’s solution (Alsever’s solution prepared according to laboratory’s standard operating procedure (SOP) into a conical graduated centrifuge tube and centrifuge at 1 000xg (speed of the centrifuge) for 5 minutes.
2. Remove supernatant.
3. Re-suspend the cells in Veronal buffer (VB) by gentle agitation and centrifuge at 1 000xg for 5 minutes.
4. Remove supernatant and buffy coat layer.
5. Remove supernatant and repeat Step 3.
6. For final centrifugation centrifuge at 1 000xg for 10 minutes.
7. Supernatant on final washing must be crystal clear and colorless.
8. Remove supernatant to prepare 3% suspension from packed red blood cells (RBC).
B. Standardization of a 3% RBC Suspension (using one of the following methods):

(i) **Wintrobe Tube Method**
1. Prepare a 20% RBC suspension in VB from washed cells as in A.
2. Mix thoroughly but gently.
3. With the aid of a Pasteur pipette fill a Wintrobe tube to the 10-graduation mark.
4. Centrifuge for 10 minutes at 1 000xg.
5. Read packed cell volume (PCV)
6. In order to prepare a 3% RBC suspension, use the following formula to calculate the volume of VB to add to a chosen volume of the original 20% suspension e.g.

\[
\text{PCV} = 18 \\
\text{Chosen volume (20% suspension) = 20ml} \\
\text{Vol. (20% suspension) x PCV} \times (\text{Vol. 20% susp.}) = \text{Vol. VB} \\
\frac{20 \times 18 - 20}{3} = 100 \text{ ml}
\]

Thus add 100ml VB to 20ml of 20% suspension to give a 3% RBC suspension.

(ii) **Calibration of the spectrophotometer**

a) A 3% suspension contains 0.96 g haemoglobin (Hb) per 100 ml.

b) All readings are done at 541 nm wavelength.

c) Allow the machine to warm up for 15 min.

d) Bring *cyanmethaemoglobin* standard to room temperature.

e) Take the reading of this standard, the optical density (OD), on the spectrophotometer using Drabkin's reagent as a blank.

f) Calculate the OD for a 3% RBC suspension diluted 1/16 in Drabkin's reagent from the following formula:

\[
\text{OD (3% suspension)} = \frac{0.96 \times \text{OD (standard)} \times 1000}{\text{Concentration of standard (mg/100 ml)} \times 16}
\]

g) Prepare a ± 4% RBC suspension from washed, packed cells.

h) Mix 1 ml of this suspension with 15 ml Drabkin's reagent.

i) Allow to stand for 10 min and then read OD.

j) Make a 3% RBC suspension from the original 4% RBC suspension by applying the following formula:

\[
\text{Vol. (veronal buffer to be added)} = \frac{\text{OD(4%)} - \text{OD(3%)} \times \text{Vol.(4%)} \times \text{OD(3%)}}{1}
\]

E.g. If the OD calculated in step 6 above is 0.5 and the OD of the 4% suspension is 0.58 and the volume of the 4% suspension to be diluted is 80 ml then:
Vol. (veronal buffer to be added) = \( \frac{0.58 - 0.5 \times 80}{0.5 \times 1} \)

Thus add 12.8 ml veronal buffer to 80 ml of 4% RBC suspension and this will give a 3% RBC suspension.

k) Lyse 1 ml of the 3% RBC suspension with 15 ml Drabkin’s reagent and read the OD. It should equal the OD calculated in step f)

l) As distilled water and not Drabkin’s reagent will be used routinely to lyse the RBC suspension, the final target OD (TOD) is obtained by lysing 1 ml of this 3% RBC suspension with 15 ml of distilled water and reading the OD using distilled water as a blank.

m) This is the target OD used in daily calculation. A table is made for routine calculation of 3% RBC (Refer to Table 1 below):
Table 1. Routine calculation for the preparation of a 3% SRBC suspension.
If the target OD (TOD) is 0.50 the following formula is used to calculate the volume of veronal to be added to an initial 100ml 4% suspension: \( ((\text{OD}^4\%\text{-0.50})/0.50) \times 99 \)

<table>
<thead>
<tr>
<th>OD4% (TOD=0.50)</th>
<th>Volume Veronal to be added to 99 ml 4% RBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.51</td>
<td>2</td>
</tr>
<tr>
<td>0.52</td>
<td>4</td>
</tr>
<tr>
<td>0.53</td>
<td>6</td>
</tr>
<tr>
<td>0.54</td>
<td>8</td>
</tr>
<tr>
<td>0.55</td>
<td>10</td>
</tr>
<tr>
<td>0.56</td>
<td>12</td>
</tr>
<tr>
<td>0.57</td>
<td>14</td>
</tr>
<tr>
<td>0.58</td>
<td>16</td>
</tr>
<tr>
<td>0.59</td>
<td>18</td>
</tr>
<tr>
<td>0.6</td>
<td>20</td>
</tr>
<tr>
<td>0.61</td>
<td>22</td>
</tr>
<tr>
<td>0.62</td>
<td>24</td>
</tr>
<tr>
<td>0.63</td>
<td>26</td>
</tr>
<tr>
<td>0.64</td>
<td>28</td>
</tr>
<tr>
<td>0.65</td>
<td>30</td>
</tr>
<tr>
<td>0.66</td>
<td>32</td>
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<tr>
<td>0.67</td>
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<td>0.68</td>
<td>36</td>
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<tr>
<td>0.69</td>
<td>38</td>
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<tr>
<td>0.7</td>
<td>40</td>
</tr>
<tr>
<td>0.71</td>
<td>42</td>
</tr>
<tr>
<td>0.72</td>
<td>44</td>
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<td>0.73</td>
<td>46</td>
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<tr>
<td>0.74</td>
<td>48</td>
</tr>
<tr>
<td>0.75</td>
<td>50</td>
</tr>
</tbody>
</table>

(iii) An alternative method for calibrating the spectrophotometer
In the absence of facilities for the cyanmethaemoglobin method the spectrophotometer may be standardised as follows:

1. Prepare a 3% SRBC suspension as follows:
   Standardisation of sheep erythrocytes
1.1 Take up to 10ml of sheep blood, stored in Alsevers solution, and add about 40ml of diluent: mix well, then centrifuge to deposit the erythrocytes.
1.2 Discard the supernatant, re-suspend the erythrocytes in about 40ml of diluent and centrifuge again.

1.3 Discard the supernatant, re-suspend the erythrocytes in about 40ml of diluent and centrifuge again.

1.4 Discard the supernatant, re-suspend the erythrocytes, this time in a 15ml graduated centrifuge tube and centrifuge at exactly 1,500xg for 10 minutes.

1.5 Note the volume of the deposit accurately, discard the supernatant, then suspend the whole of the deposit in 32.3 times its volume of CF diluent to produce a 3% suspension. For reproducible results it is recommended that sufficient erythrocytes be washed to give a final deposit of about 1 to 2ml

2. Add 1 ml of this suspension to 15 ml distilled water and mix.

3. As soon as lysis is complete, measure the OD.

4. Repeat steps 1, 2 and 3 several times and calculate the mean OD. This is the target OD used in daily calculation (See table above).

* Cyanmethaemoglobin can be obtained from BDS International Diagnostics GmbH
Email: Info@BDS-International.de
Item Code: 0325-006 Hemoglobin Standard (20g/dl) 6x 15ml

Spectrophotometrical Method

1. Prepare an initial 4% suspension by taking 4ml packed SRBC (from the washing procedure) and 96ml VB.

2. Prepare from this 4% suspension a 1:16 dilution by adding 1ml of 4% SRBC to 15ml distilled water. Leave a moment in order to completely lyse the RBCs.

3. Use two cuvettes: one filled with distilled water (to use as Blank), and the second to fill with the 1:16 dilution of lysed RBCs.

4. Read the absorbance value at 541 nanometer (nm): Each laboratory shall calibrate their instrument with each batch of blood collected.

5. Use the following formula to calculate the volume of VB to add to the suspension to correct the OD to 0.5 if necessary:

\[
\text{OD reading of initial 4% - Target OD} \times \frac{\text{The volume that is left after 1ml has been taken out to make 1:16}}{\text{Target OD}}
\]

\[
\frac{\text{OD reading (4%) – 0.5}}{0.5} \times \frac{\text{The volume that is left after 1ml has been taken out to make 1:16}}{0.5}
\]

For example:
If the OD reading was 0.58, the formula would be applied as follows:

\[
0.58 - 0.5 = 15.8 \text{ml (round off to 16ml)}
\]

Thus, 16ml VB must be added to the initial 4% suspension to make it spectrophotometrically a 3% suspension.
C. Method for titration of Haemolysin (Amboceptor)

1. Set up 2 racks of 10 tubes (11 x 75 mm), one for the serial dilution of haemolysin and the other for the titration.

2. Prepare a 3% RBC suspension in veronal buffer.

3. Prepare a 1/1000 dilution of haemolysin in veronal buffer (0,1 ml haemolysin in 99,9 ml buffer).

4. Make further dilutions of haemolysin in the first rack according to the following schedule:

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Dilution</th>
<th>1/1000 Master dilution (ml)</th>
<th>Veronal buffer (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1/1000</td>
<td>1,0</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>1/2000</td>
<td>1,0</td>
<td>1,0</td>
</tr>
<tr>
<td>3</td>
<td>1/3000</td>
<td>0,5</td>
<td>1,0</td>
</tr>
<tr>
<td>4</td>
<td>1/4000</td>
<td>0,5</td>
<td>1,5</td>
</tr>
<tr>
<td>5</td>
<td>1/5000</td>
<td>0,5</td>
<td>2,0</td>
</tr>
<tr>
<td>6</td>
<td>1/6000</td>
<td>0,5</td>
<td>2,5</td>
</tr>
<tr>
<td>7</td>
<td>1/7000</td>
<td>0,5</td>
<td>3,0</td>
</tr>
<tr>
<td>8</td>
<td>1/8000</td>
<td>0,2</td>
<td>1,4</td>
</tr>
<tr>
<td>9</td>
<td>1/9000</td>
<td>0,2</td>
<td>1,6</td>
</tr>
<tr>
<td>10</td>
<td>1/10000</td>
<td>0,2</td>
<td>1,8</td>
</tr>
</tbody>
</table>

5. In addition 2 control tubes are set up as follows:

   Tube No. 11  Complement 1/20  0,4 ml
               3% RBC suspension 0,4 ml
               Veronal buffer  1,2 ml

   Tube No. 12  Haemolysin 1/1000 0,4 ml
               3% RBC suspension 0,4 ml
               Veronal buffer  1,2 ml

These 2 tubes pass through the same process as the titration tubes, as set out below, and neither must show any sign of haemolysis.

6. Mix each dilution starting with the highest dilution (Tube No. 10) and transfer 0,4 ml to the appropriate tube in the titration rack.

7. Add 0,4 ml of the 3% RBC suspension to each tube and **mix immediately**.

8. Shake and place in a water bath at 37°C for 30 min to sensitize.

9. Add 0,4 ml of complement with a final dilution of 1/20 to each tube.

10. Add 0,8 ml veronal buffer to each tube.

11. Shake and incubate for 30 min in a water bath a 37 °C.
12. Remove from the water bath and read.
13. The unit of haemolysin referred to as 1 MHD (minimal haemolytic dose) is the highest dilution showing complete haemolysis.
14. Use 5 MHDs in the CFT.

Note Haemolysin with a titre of less than 1/2000 should not be used.

*Calculation of 5 MHDs*

If the haemolysin titration is 1/2000. Then 1 MHD is contained in a 1/2000 dilution. Thus 5 MHDs are contained in a 5/2000 = 1/400 dilution. Therefore 0,25/100 is the correct dilution for 100 ml to contain 5 MHDs. Thus to supply 5 MHDs in 100 ml veronal buffer one must add 0,25 ml of haemolysin with a titre of 1/2000.

8.2.2 Preparation of Sensitised Haemolytic System

Equal volumes of appropriately diluted amboceptor (5MHD’s) in veronal buffer is mixed with the 3% SRBC suspension to make up the required volume needed for the test run by pouring simultaneously into a third container and agitating for a short while. The haemolytic system is put into a water bath at 37°C for 30 minutes at 37°C to allow the sensitisation. After 15 minutes the RBCs can be mixed gently.

8.3 Inactivation of test serum

8.3.1 Prepare a 96-well microtitre plate filled with 1.2ml polypropylene cluster tubes in the test procedure format for the day.
8.3.2 Test serum should be clear otherwise centrifuged before being poured into the cluster tubes.
8.3.3 Inactivate the test and control sera in a water bath, at 60°C±2°C for 30 minutes. It is important that the water level covers the level of the serum.
8.3.4 The sera should be allowed to cool down before commencing with the test.

8.4 Titration of complement

1. Prepare the haemolytic system (sensitised sheep red blood cells).
2. Prepare a 1/40 dilution of complement in cold diluent (veronal buffer).
3. Dispense the 1/40 dilution of complement into 10 tubes in the quantities shown in Table 2.

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Complement 1/40 (ml)</th>
<th>Diluent (ml)</th>
<th>Sensitised sheep red blood cells (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.03</td>
<td>0.72</td>
<td>0.25</td>
</tr>
<tr>
<td>2.</td>
<td>0.04</td>
<td>0.71</td>
<td>0.25</td>
</tr>
<tr>
<td>3.</td>
<td>0.05</td>
<td>0.7</td>
<td>0.25</td>
</tr>
<tr>
<td>4.</td>
<td>0.06</td>
<td>0.69</td>
<td>0.25</td>
</tr>
<tr>
<td>5.</td>
<td>0.075</td>
<td>0.675</td>
<td>0.25</td>
</tr>
<tr>
<td>6.</td>
<td>0.1</td>
<td>0.65</td>
<td>0.25</td>
</tr>
<tr>
<td>7.</td>
<td>0.125</td>
<td>0.625</td>
<td>0.25</td>
</tr>
<tr>
<td>8.</td>
<td>0.15</td>
<td>0.6</td>
<td>0.25</td>
</tr>
<tr>
<td>9.</td>
<td>0.2</td>
<td>0.55</td>
<td>0.25</td>
</tr>
<tr>
<td>10.</td>
<td>0.25</td>
<td>0.5</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Thirty minutes in the water bath at 37°C
4. Add the appropriate quantities of diluent, also shown in the Table.
5. Shake the tubes to mix the contents and place in the water bath at 37°C for 30 minutes.
6. Add 0.25 ml of the haemolytic system to all the tubes, mix well and replace in the water bath at 37°C for another 30 minutes.
7. Remove the tubes from the water bath and read the result. The quantity of complement in the first tube to show complete haemolysis is 1 exact unit and the quantity in the next tube containing more complement, 1 full unit; 2 full units are used in the diagnostic test. Calculate the dilution of complement to use in the diagnostic test using the formula:

\[
\frac{40}{4 \times 2 \text{ full units}}
\]

For example, if haemolysis is absent or incomplete in tubes 1 to 4, and complete in tubes 5 to 10, the exact unit is 0.075 and the full unit is 0.1, then the dilution factor will be:

\[
\frac{40}{4 \times 0.2} = 50
\]

i.e. a dilution of 1/50 will be used in the diagnostic test.

8.5 Titration of Antigen

Serial dilutions of the antigen are made e.g. 1/100, 1/200, 1/300, etc. A positive serum of low to medium titre is selected or prepared. A single microtitration plate is used as in the example below (Table 3).

### Table 3. An example of a chequerboard titration of antigen.

<table>
<thead>
<tr>
<th>Dilutions of medium titre serum</th>
<th>Antigen dilutions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/100 1/200 1/300 1/400 1/500 1/600 1/700 1/800</td>
</tr>
<tr>
<td>Row</td>
<td>1 2 3 4 5 6 7 8</td>
</tr>
<tr>
<td>A</td>
<td>Used for the deposition of a medium titre serum</td>
</tr>
<tr>
<td>1/2</td>
<td>B* - - - - - - -</td>
</tr>
<tr>
<td>1/4</td>
<td>C 4+ 4+ 4+ 4+ 4+ 3+ 2+ 2+</td>
</tr>
<tr>
<td>1/8</td>
<td>D 4+ 4+ 4+ 3+ 2+ - - -</td>
</tr>
<tr>
<td>1/16</td>
<td>E 4+ 4+ 3+ - - - - -</td>
</tr>
<tr>
<td>1/32</td>
<td>F 2+ 4+ - - - - - -</td>
</tr>
<tr>
<td>1/64</td>
<td>G - 1+ - - - - - -</td>
</tr>
<tr>
<td>1/128</td>
<td>H - - - - - - - -</td>
</tr>
</tbody>
</table>

* Anti-complementary row

4+ = 0% haemolysis; 3+ = 25% haemolysis; 2+ = 50% haemolysis; 1+ = 75% haemolysis; - = 100% haemolysis
In the example, the highest titre is recorded for the antigen dilution of 1/200 and this is the dilution to be used. Any anti-complementary activity in the antigen is detected by a similar titration using a negative serum.

8.6 Test Procedure

1. The test is carried out in 96-well, U-bottom microtitre plates. See Figure for orientation of plate.
2. Two wells, one for each, will be used for positive and negative controls on each plate.
3. Add 25µl VBS to wells 1 – 8 of row A-H.
4. Transfer 25µl of inactivated sample from the inactivation plate to the wells in column 8 in rows A-F and dispense 25µl of positive control serum to the well in column 8 row H and 25µl negative control serum to the well in column 8 row G. Take 25µl of serum from the wells A-H in column 8 and make serial doubling dilutions from column 1-7. Discard the last 25µl.
5. Add 25µl B. ovis antigen at working dilution to columns 1-7, row A-H, rows A-H and an additional 25µl VB to column 8 rows A-H.
6. Add 25µl Complement at working dilution to wells 1-8 of rows A-H.
7. Optional: Mix equal volumes (just before use) of B. ovis antigen and complement and add 50 µl as one step to all wells except the anti-complementary row. **NB! Remember to add separate complement to the anti-complementary row!!**
8. Incubate plates at 37°C for 30 minutes. Plates should not be stacked to allow for uniform heat distribution.
9. After 30 minutes add 50µl of haemolytic system to wells 1-8 rows A-H.
10. Place plates on a shaker and incubate for a further 30 minutes at 37°C. *(OIE SAYS 10 MIN)*
11. Plates are read over a magnifying mirror or X-ray viewing box.

---

**Figure 1. Orientation of the microtitre plate**

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tbody>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>B</td>
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8.7 Results

1. A negative result is where no sedimentation of SRBC is present, i.e. complete haemolysis.
2. A positive result is where sedimentation of SRBC is present as a distinct button at the bottom of the well, and is scored between 1 and 4. The final dilution is determined where 50% sedimentation occurs in a dilution and is converted to international units/ml (IUml⁻¹) using the prescribed conversion chart. (Table 4) (Differences of two scores higher and lower in the positive control serum will be accepted).
3. Anti-complementary row should, otherwise the result for that particular test plate is not valid.
4. Positive and negative control on each plate should give correct titre.

Table 4. The conversion of CFT endpoint reactions to IU⁻¹ on a scale where 50% haemolysis in a 1/220 serum dilution is equivalent to 1000 IU ml⁻¹

| Row | Serum Dilution<sup>a</sup> | Final Dilution<sup>b</sup> | Endpoint Reading | Reaction | IU ml<sup>c</sup><sup>|</sup> | SAU ml<sup>d</sup> |<sup>|</sup>
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<tr>
<td>C</td>
<td>1/4</td>
<td>1/20</td>
<td>100</td>
<td>0 or-</td>
<td>0</td>
<td>15</td>
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<td></td>
<td></td>
<td></td>
<td>75</td>
<td>1 or +</td>
<td>2</td>
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<td></td>
<td></td>
<td>50</td>
<td>2 or ++</td>
<td>18</td>
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<td></td>
<td>25</td>
<td>3 or +++</td>
<td>21</td>
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<td></td>
<td>0</td>
<td>4 or ++++</td>
<td>24</td>
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<td>D</td>
<td>1/8</td>
<td>1/40</td>
<td>75</td>
<td>1 or +</td>
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<td>50</td>
<td>2 or ++</td>
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<td>4 or ++++</td>
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<td>1/16</td>
<td>1/80</td>
<td>75</td>
<td>1 or +</td>
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<td>1/32</td>
<td>1/160</td>
<td>75</td>
<td>1 or +</td>
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<td>G</td>
<td>1/64</td>
<td>1/320</td>
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<td>1 or +</td>
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<td>25</td>
<td>3 or +++</td>
<td>344</td>
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<td></td>
<td>0</td>
<td>4 or ++++</td>
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<td>H</td>
<td>1/128</td>
<td>1/640</td>
<td>75</td>
<td>1 or +</td>
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<td>50</td>
<td>2 or ++</td>
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<td></td>
<td>25</td>
<td>3 or +++</td>
<td>688</td>
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<td>0</td>
<td>4 or ++++</td>
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<sup>a</sup> Serum dilution = dilution factor with veronal buffer only
<sup>b</sup> Final dilution = final dilution factor after all reagents are added
<sup>c</sup> IU ml⁻¹ = International Units per millilitre
<sup>d</sup> SAU ml⁻¹ = South African Units per millilitre

9. QUALITY CONTROL

Positive and negative controls must be run with each plate of serum tested.
10. **WASTE DISPOSAL**

Samples are discarded into biohazard waste bags destined for incineration or for removal by an approved waste removal service provider (Refer to as per Waste Disposal SOP).

11. **FORMS**

All raw data forms pertaining to the test are to be filed and retained for a minimum of 5 years.

12. **REPORTING**

12.1 Copies of reports are to be kept for 5 years.
12.2 Original test results are sent to the relevant State Veterinarian.

13. **REFERENCES**

13.2 Management of laboratory facilities (ISO clause 4).
13.3 Safe handling of biological specimens and chemicals (ISO clause 5.8).
13.4 Outside services and supplies (ISO clause 4.6).
13.7 SANAS R57-document Criteria for accreditation of Veterinary Serology.
13.8 SANAS R25 – document Criteria for calibration and verification checks of equipment used in accredited facilities.
13.9 SANAS R63 – document Guidelines for transport of biological Veterinary specimens