NATIONAL STANDARD METHOD

COMPLEMENT FIXATION TESTS

VSOP 18

Issued by Standards Unit, Department for Evaluations, Standards and Training
Centre for Infections

COMPLEMENT FIXATION TESTS
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STATUS OF NATIONAL STANDARD METHODS

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Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

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COMPLEMENT FIXATION TESTS

SCOPE OF DOCUMENT

This National Standard Method describes the procedure for performing complement fixation tests (CFT) on serum specimens.

INTRODUCTION

Background

Response to infection may be demonstrated by the development of or increase in the levels of a specific antibody to the causative agent between two specimens; one taken in the acute phase of illness and the other taken in the convalescent phase. The CFT is the commonest test used to demonstrate this increase in antibody levels against a wide range of viruses. CFT may also be used to detect the presence of intrathecal antibody in CNS infection.

Patient's serum, in which the naturally occurring complement (C') has been inactivated, is mixed with standard antigens and Guinea Pig complement (GPC'). The GPC' is fixed in the reaction between the antigen and any antibody in the patient's serum. Absence of antibody leaves the added complement unfixed. The addition of an indicator system, consisting of sheep red blood cells sensitised with a haemolysin (specific antibody to sheep red blood cells), to the reaction well enables any residual complement to be detected and is visualised by the lysis of the sheep red blood cells. Absence of available complement and therefore presence of antibody in the patient's serum is visualised by the sheep red blood cells remaining intact.

Careful standardisation and control of the reagents permits the demonstration of rising titres of antibody between the acute and convalescent specimens as evidence of recent infection.
1 SAFETY CONSIDERATIONS

1.1 SPECIMEN COLLECTION
Appropriate hazard labelling according to local policy.
Specimens may contain blood borne viruses.

1.2 SPECIMEN TRANSPORT AND STORAGE
Compliance with current postal and transportation regulations is essential.

1.3 SPECIMEN PROCESSING
Specimens received in the laboratory may contain blood borne viruses. Blood specimens should be processed using universal precautions. Serum separation should be carried out wearing gloves and eye protection.

1.4 CHEMICAL HANDLING
Veronal Buffered Saline (VBS) contains barbiturates and must be stored and handled safely.

Stock reagents and chemicals must be stored in a locked chemical storage cupboard.

Sodium azide in VBS should be handled carefully as sodium azide can form metallic azides with metal plumbing that is explosive if roughly handled. Disposal of azide containing reagents into sink waste systems should be carried out using large volumes of water. Alternatively, Bronidox may be used which can have a mildly corrosive action on some metals, so reagents containing Bronidox should not be stored in unprotected metal containers.

The above guidance should be supplemented with local COSHH and risk assessments

2 SPECIMEN COLLECTION

2.1 TYPES OF SPECIMEN
Serum

2.2 OPTIMAL TIMING OF SPECIMEN COLLECTION
Specimens of clotted blood should be collected as early in the acute phase of the illness as possible and a further specimen collected in the convalescent phase of the illness approximately 10-14 days after the acute specimen. In some circumstances where an acute specimen has not been obtained it can still be worthwhile to obtain a convalescent phase specimen. Bacterial contamination of the specimen should be avoided. If collecting CSF for intrathecal antibody detection a serum sample should be collected at the same time to allow a comparison of antibody titres and albumin index determination. It is important to control blood contamination of CSF.

2.3 CORRECT SPECIMEN TYPE AND METHOD OF COLLECTION
N/A

2.4 ADEQUATE QUANTITY AND APPROPRIATE NUMBER OF SPECIMENS
N/A
3 SPECIMEN TRANSPORT AND STORAGE

3.1 TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING
Specimens should be transported to the laboratory as quickly as possible and serum separated from the clot as soon as possible after receipt.

3.2 SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION
Ideally, serum should be aliquotted to two storage vials, one stored at +4°C for immediate use and the other frozen at -20°C or below. In addition, an aliquot can be stored at -20°C until much later ie after testing is complete.

4 EQUIPMENT AND REAGENTS

4.1 EQUIPMENT
- U-well microtitre plates
- Microtitre plate covers
- Multi-channel pipettes (25 µL)
- 25 µL “dropper” disposable pipettes
- Stepper pipettes (25 µL)
- +56°C Waterbath
- +37°C Incubator
- +37°C Waterbath
- Light Box (optional)
- Plate shaker
- +4°C refrigerator
- Sterile Universal containers
- Sterile Bijoux bottles
- Plastic test tubes (75 x 12 mm)
- Storage vials
- Robotic Microtitre plate processor (eg Kemble)
- 10 mL and 1 mL pipettes
- Haematocrit tubes

4.2 REAGENTS
- GPC'
- Sheep red blood cells (SRBC)
- Sheep red blood cell haemolysin (amboceptor)
- Veronal Buffered Saline (VBS)
- CFT antigens
- CFT control antisera
- Sodium azide/VBS
Sterile distilled water

5 SPECIMEN PROCESSING

5.1 STANDARDISATION OF REAGENTS

It is important to determine the optimal dilution or concentration of CFT reagents ie antigen, antiserum controls, GPC and haemolysin.

5.1.1 COMPLEMENT AND HAEMOLYSIN CHESSBOARD TITRATION

To determine the optimal concentrations of GPC and haemolysin, a chessboard, or two-dimensional titration, is performed.

Dilutions of GPC are prepared at 20% differences in concentration (see Fig. 1) to obtain an accurate end point. It is important to avoid frothing.

Freeze dried (lyophilised) GPC is reconstituted using sterile distilled water to the stated volume on the vial. This may be stored at 4°C for up to 6 days or frozen in aliquots at -70°C. Thaw out just before use.

Note: Richardson's preserved GPC is hypertonic; therefore the 1/10 dilution is achieved by adding 7 mL distilled water to 1 mL of reconstituted complement.

In labelled universal containers, prepare dilutions as shown in Fig. 1:

<table>
<thead>
<tr>
<th>Dilution</th>
<th>1/30</th>
<th>1/38</th>
<th>1/47</th>
<th>1/59</th>
<th>1/73</th>
<th>1/92</th>
<th>1/114</th>
<th>1/143</th>
<th>1/179</th>
<th>1/224</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled Water</td>
<td>3.5</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Reconstituted Complement</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VBS</td>
<td>Add 8 mL</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
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<tr>
<td></td>
<td>Mix</td>
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<td>Mix</td>
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<td>Mix</td>
<td>Mix</td>
<td>Mix</td>
<td>Mix</td>
<td>Mix</td>
</tr>
<tr>
<td></td>
<td>Transfer 8 mL to next tube</td>
<td>Transfer 8 mL to next tube</td>
<td>Transfer 8 mL to next tube</td>
<td>Transfer 8 mL to next tube</td>
<td>Transfer 8 mL to next tube</td>
<td>Transfer 8 mL to next tube</td>
<td>Transfer 8 mL to next tube</td>
<td>Transfer 8 mL to next tube</td>
<td>Discard 8 mL</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: Procedure for performing GPC dilutions
Label a microtitre plate as illustrated in Fig. 2:

<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>
</tr>
</thead>
<tbody>
<tr>
<td>Haemolytic serum dilutions</td>
<td>Complement</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/30</td>
<td>1/38</td>
<td>1/47</td>
<td>1/59</td>
<td>1/73</td>
<td>1/92</td>
<td>1/114</td>
<td>1/143</td>
<td>1/179</td>
<td>1/224</td>
<td>control</td>
<td></td>
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<tr>
<td>25</td>
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<td></td>
<td></td>
<td>A</td>
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<td>50</td>
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<td>B</td>
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<td>100</td>
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<td></td>
<td>C</td>
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<td>200</td>
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<td>400</td>
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<tr>
<td>800</td>
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<td></td>
<td></td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>G</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 2**: Template for setting up haemolysin/GPC' chessboard titration

- To each well add 50 µL (2 volumes) of VBS. Add 25 µL (1 volume) of each dilution of GPC' to the appropriate column of wells. Add an additional volume (25 µL) of VBS to column 11.
- Gently mix, cover with a microtitre plate cover and place at +4°C overnight.
- In labelled 75x12 mm test tubes, prepare 1 mL volumes of doubling dilutions of haemolysin in VBS from 1/25 to 1/800. Add 1 mL of VBS to a tube labelled Control.
- Prepare 4% SRBCs in VBS (see Appendix 1) and add 1 mL to each of 7 labelled bijoux bottles.
- Mix 1 mL of the appropriate dilution of haemolysin (see Appendix 1) or control with the corresponding 1 mL of sheep red blood cells.
- Replace the caps on the bijoux bottles, mix gently and place at +4°C overnight.

When overnight incubation is complete:

- Incubate the plate at +37°C. Remove the bijoux bottles containing the now sensitised SRBCs (HS), resuspend the cells by gentle mixing and incubate at +37°C (in a waterbath).

After 30 minutes remove the plate and the sensitised cells and after resuspending the cells by gentle mixing, add 25 µL of the appropriate sensitised cells to the appropriate row of the microtitre plate. Gently tap the plate or use a plate mixer to mix the cells and return the plate to +37°C for 30 minutes. Repeat the mixing after 10 and 20 minutes and when removed from the incubator. Place the covered plate at +4°C for 2 hours to allow the cells to settle or centrifuge.

Remove the plate from the refrigerator and read using a light box if required. Score the remaining cells / degree of haemolysis on a scale of 0 to 4 where:

- Total lysis – no cells remaining = 0
- 1 – 24% cells remaining = Trace
- 25% cells remaining = 1
- 50% cells remaining = 2
- 75% cells remaining = 3
- 100% cells remaining = 4
Typical chessboard results are shown in Fig. 3:

<table>
<thead>
<tr>
<th>Haemolytic serum</th>
<th>Complement</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/38</td>
<td>1/47</td>
</tr>
<tr>
<td>A 1/25</td>
<td>0</td>
</tr>
<tr>
<td>B 1/50</td>
<td>0</td>
</tr>
<tr>
<td>C 1/100</td>
<td>0</td>
</tr>
<tr>
<td>D 1/200</td>
<td>0</td>
</tr>
<tr>
<td>E 1/400</td>
<td>0</td>
</tr>
<tr>
<td>F 1/800</td>
<td>0</td>
</tr>
<tr>
<td>G Control</td>
<td>4</td>
</tr>
</tbody>
</table>

**Figure 3**: Example of expected results in a haemolysin/GPC' chessboard titration

The Optimum Sensitising Concentration (OSC) of the haemolysin is that dilution which gives the most lysis with the highest dilution of C' (see Fig. 4). In the above example the OSC is 1/100 and the haemolysin should be used at that dilution.

The Haemolytic Dose giving 50% lysis (HD50) of the Complement is that dilution which gives 50% lysis (a reading of 2) with the OSC of haemolysin (see Fig. 4). In the above example the HD50 is 1/143. In the test proper, the Complement is used at 3HD50 ie 1/143 x 3 = 1/47.
5.1.2 ANTIGEN CHESSBOARD TITRATION

Chessboard titrations are carried out on all new batch numbers of antigens. Select 6 dilutions at which to test the new antigen eg if the vial suggests an optimal dilution of 1:40 then make 1:10, 1:20, 1:30, 1:40, 1:50 and 1:60 dilutions. Make at least 600 µL of each dilution using VBS azide (see Appendix 2).

Two microtitre plates are needed for the chessboard titration:

- One plate for the positive control (antiserum) and C' back titration (see Fig. 5). Select a serum that has a given good titre against the particular antigen (preferably with a known endpoint eg 1/64 or 1/128). In addition, select a known negative serum. Make 1/16 dilutions and inactivate at 56°C for 30 minutes.

- Another plate for the two patient specimens. The positive and negative specimens should be selected on the titre obtained against the particular antigen (ie with a known endpoint of 1/64 or 1/128) (see Fig. 6). Make 1/16 dilutions and inactivate at 56°C for 30 minutes.

Figure 4: Photograph of expected haemolysin/GPC' chessboard titration
Figure 5: Example of a template for setting up an antigen/positive control titration

Figure 5 shows the procedure for setting up an antigen titration using a positive antiserum control:

- To columns 2-6, rows A-F, add 25 µL VBS (column 6 is the serum control)
- To column 6, rows A-F, add a further 25 µL VBS in place of the antigen
- To columns 1, 2 and 6, add 25 µL of the appropriate antiserum. Double dilute from columns 2-5
- To columns 1-5, rows A-F, add 25 µL of the appropriate antigen dilution
- Add 25 µL 3HD50 GPC' to columns 1-6, rows A-F. Tap the plate to mix the reactants and incubate +4°C overnight

To set up a GPC' back titration:

- Add 25 µL VBS and appropriate antigen dilution (50 µL in total) to columns 10-12, rows A-F. Tap the plate to mix the reactants
- To column 10, rows A-F, add 25 µL 3HD50 GPC', to column 11 add 25 µL 1HD50 GPC' and to column 12 add 25 µL ½HD50 GPC'
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**Antigen dilution**

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<tr>
<td>10</td>
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</tr>
</tbody>
</table>

Positive patient specimen  
Negative patient specimen

**Figure 6**: Example of a template for setting up an antigen/patient serum specimen titration

Figure 6 shows how to set up the second plate using positive and negative patient’s serum. The procedure for each specimen is carried out in the same way as the standard positive control antiserum (see Fig. 5):

- Add 25 µL of 3HD50 GPC’ to columns 1-12, rows A-H. Tap plates to mix reactants. Incubate at +4°C overnight

After overnight incubation, incubate the two microtitre plates at +37°C for 30 minutes. At the same time, incubate a sufficient volume of 4% sensitised SRBCs or haemolytic system (HS) (see Appendix 1) at +37°C (in a waterbath) for 30 minutes.

Add 25 µL of HS to all wells in use and incubate at +37°C for 30 minutes. Mix the reactants in the microtitre plates after 10, 20 and 30 minutes. Incubate at +4°C for 2 hours. Read the plate.

The optimal dilution of the antigen is that which gives the highest titre for the positive antiserum control and which additionally gives the predetermined titre for the positive patient specimen. The dilution should also show a normal pattern on the complement back titration and a negative result should be obtained with the negative patient control.

### 5.1.3 Antiserum (Positive Control) Chessboard Titration

A chessboard titration is carried out on all new batch numbers of antisera.

Reconstitute the antisera, according to manufacturer’s instructions.

Use manufacturer’s suggested optimal dilution as a guide to chessboard dilutions:

If the suggested optimal dilution of antisera is, for example 1:40, then make a series of dilutions in VBS/azide, as follows: 1:20, 1:30, 1:35, 1:40, 1:45, 1:50, 1:55, 1:60. Make at least 600 µL of each dilution using VBS azide (see Appendix 2).

Using a microtitre plate (see fig. 7), the chessboard titration is carried out as follows:
Antiserum dilutions | Appropriate antigen | S/C | Antigen back titration | C' back titration
<table>
<thead>
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<td></td>
<td></td>
<td>1</td>
<td>3HD50</td>
<td>1HD50</td>
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<tr>
<td>20</td>
<td>A</td>
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<tr>
<td>30</td>
<td>B</td>
<td></td>
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<tr>
<td>35</td>
<td>C</td>
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<td>40</td>
<td>D</td>
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<td>45</td>
<td>E</td>
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<tr>
<td>50</td>
<td>F</td>
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<tr>
<td>55</td>
<td>G</td>
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<tr>
<td>60</td>
<td>H</td>
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Figure 7: Template for setting up an antiserum/antigen titration

- Add 50 µL of the appropriate antiserum dilution to column 1. Add 25 µL VBS into columns 2-6, rows A-H
- Double dilute antiserum in each of rows A-H from columns 1-5. Add 25 µL of the appropriate antiserum dilution to column 6. (Column 6 is the serum control)
- Add 25 µL of the appropriate antigen at its current working dilution to columns 1-5, rows A-H. Tap plate, to mix reactants
- Add 25µL of 3HD50 C’ into wells 1-6, rows A-H. Tap plate to mix reactants and incubate at +4°C overnight

In addition, antigen and C’ back titrations are set up (see Fig. 7):

- Make up 3, 1, ½ HD50 complement
- Antigen back titration - place 25 µL of VBS into wells 7, 8 and 9 in row A. Place 25 µL of antigen at its working dilution into each of the three wells. Place 25 µL of 3HD50 complement into column 7, row A, 25 µL of 1HD50 complement into column 8, row A and 25 µL of ½HD50 complement into column 9, row A
- C’ back titration - place 50 µL of VBS into wells 10, 11 and 12 in row A. Place 25 µL of 3HD50 complement into column 10, row A, 25 µL of 1HD50 complement into column 11, row A and 25 µL of ½HD50 complement into column 12, row A

When the overnight incubation is complete, place plates at +37°C for 30 minutes. At the same time, incubate a sufficient volume of HS at +37°C (in a waterbath) for 30 minutes (see Appendix 1). Add 25 µL of HS to all wells. Incubate at +37°C for 30 minutes and mix after 10, 20 and 30 minutes. Incubate at +4°C for 2 hours. Read the plates (see Fig. 8).

The optimum dilution of antiserum is that which gives complete fixation at the highest dilution of antiserum.

Dispense antiserum, and label containers, with all relevant details (dilution used, batch no, expiry date, date made up and antigen type).
5.2 CFT SCREENING

5.2.1 CFT PROCEDURE

The CFT is performed as follows. The antigens used are dependent on the clinical details (see Fig. 9):

DAY 1

- Prepare and label dilution tubes for patient serum specimens. Dilute sera 1/16 by adding 50 µL of serum to 750 µL of VBS in the labelled tube, then mix thoroughly. Cover tubes and inactivate diluted sera in a waterbath at 56°C for 30 minutes.
- Add 25 µL of inactivated serum to rows H,G and C (row C is the serum control). Add 25 µL VBS to rows G-C.
- Double dilute the serum from rows G-D to give a range of dilutions from 1/32 to 1/256 (see Fig. 9).
- Add 25 µL of appropriate antigens (diluted using VBS azide – see Appendix 2) to the wells ie for influenza A CFT, add antigen to column 1, rows H-D. Add 25 µL VBS to row C in place of the antigen. Tap plate to mix the reactants.
- Make up sufficient 3HD50 C' for all plates and add 25 µL to all wells. Tap plate to mix the reactants. Cover plate and incubate overnight at +4°C.
### Figure 9: Example of template for setting up a CFT

Standard antiserum controls, antigen controls and C' control should additionally be set up:

- The antiserum controls are performed using the same process as shown in Figure 9.
- Antigen controls are set up by adding 25 µL VBS to row B (see Fig. 10). Add 25 µL of the appropriate antigens into the appropriate wells. Tap plate to mix the reactants. Add 25 µL 3HD50 C' to all wells and tap the plate.
- The C' control is set up by adding 25 µL 3HD50 C' to well 10A, 25 µL 1HD50 C' to well 11A and 25 µL ½HD50 C' to well 12A. Add 50 µL VBS to all three wells.
- Cover plate and incubate overnight at +4°C.
- One volume (25 µL) of VBS should be added to replace any component that is omitted. Therefore each well will contain a final volume of 4 x 25 µL.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Specimen dilution</th>
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<tbody>
<tr>
<td></td>
<td>1/16</td>
</tr>
<tr>
<td></td>
<td>1/32</td>
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<tr>
<td></td>
<td>1/64</td>
</tr>
<tr>
<td></td>
<td>1/128</td>
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<tr>
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<td>1/256</td>
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<td></td>
<td>S/C</td>
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<td></td>
<td>C</td>
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<tr>
<td></td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>A</td>
</tr>
</tbody>
</table>

**Influenza A** | 1 |
**Influenza B** | 2 |
**Adenovirus**  | 3 |
**Chlamydia**   | 4 |
**Q2(Coxiella)**| 5 |
**RSV**         | 6 |
**Mycoplasma**  | 7 |
**Measles**     | 8 |
**VZ**          | 9 |
**HSV**         | 10 |
Figure 10: Example of template for setting up antiserum, antigen and C’ control plate

Make up HS (see Appendix 1). Test an equal vol of HS and C’ (eg 50µL of HS with 50µL of 3HD50 C’) by incubating at 37°C for 10 minutes for complete lysis. If complete lysis occurs, store overnight at 4°C.

**DAY 2**

The CFT procedure is continued as follows:

- Transfer the CFT plates to the incubator (37°C) for 30 minutes to warm up. Take the HS from the fridge and incubate in a 37°C waterbath for 30 minutes
- Add 25 µL of HS (ensuring mixing has occurred) to each well being tested. Shake the plates, either by tapping gently or on a plate shaker, to resuspend the erythrocytes
- Incubate plates at +37°C for 30 minutes, mixing reactants as much as possible
- Transfer the plates to +4°C for two hours to stop the reaction and allow any unlysed cells to settle. Transfer plates to the bench for 10 minutes to allow the reactions to be read

**5.2.2 READING OF CF TESTS**

**DAY 2**

- Read and report titres (see Fig. 11), transfer any repeats and >16 screens etc. to titration sheets ready to process as above

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Antigen back titration</th>
<th>C’ back titration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3HD50</td>
<td>1HD50</td>
</tr>
<tr>
<td>Influenza A</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Influenza B</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Adenovirus</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Chlamydia</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Q2(Coxiella)</td>
<td>5</td>
<td></td>
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<tr>
<td>RSV</td>
<td>6</td>
<td></td>
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<tr>
<td>Mycoplasma</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Measles</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>VZ</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>HSV</td>
<td>10</td>
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</tbody>
</table>
The reciprocal of the highest dilution of serum giving a reading of 2 is taken as the serum titre.

Sera showing anticomplementary activity should be treated with guinea pig serum (see Appendix 3). Anticomplementary activity is shown by the absence of lysis when only serum, C' and HS are present ie in the serum control wells (column C).

Sera showing haemagglutinating activity should be treated with sheep red blood cells (see Appendix 4).

The antigen and C' back titrations should show complete lysis in the 3HD50 C' well; 50% lysis in the 1HD50 C' well and no lysis in the ½ HD50 C' well (see Fig. 8).

The antiserum controls are diluted to give the expected titre in the 3rd well ie row F (see Fig. 12).

Figure 11: Photograph of acute and convalescent CFT
Scoring of reactions in the CF test

0 = no. of cells remaining (complete lysis)  
1 = approx. 25% cells remaining  
2 = approx. 50% cells remaining  
3 = approx. 75% cells remaining  
4 = approx. 100% cells remaining  
tr = approx. 1 – 24% cells remaining  

Influenza A  
Influenza B  
Adenovirus  
Chlamydia  
QII  
RSV  
Mycoplasma  
Mumps  
Measles  
VZV  
HSV  
QI  

Figure 12: Photograph of antiserum control CFT

Illustrates the variation around the expected titre with readings in the expected well ranging from 4 to 1.

The third row (adenovirus) also illustrates a slightly anti-complementary antiserum with a reading of 1 in the serum control.

The measles control serum is showing a two-fold higher titre than expected whilst the VZ control serum is reading two-fold lower than expected.

6 QUALITY ASSURANCE

A quality system should be in place to ensure that appropriate internal and external quality assessment and quality control procedures are maintained.

7 LIMITATIONS

Successful detection of antibody in patient’s serum depends on correct specimen collection, transport, storage and processing and the provision of adequate/suitable clinical information.

The procedure(s) in these documents aim to describe good microbiological standard methods for the specimen types specified. Other procedures may be required and professional interpretation by qualified staff is essential. Please note that knowledge of infectious diseases changes constantly and although this NSM is regularly reviewed it may not include emerging pathogens.

8 REPORTING PROCEDURE

8.1 REPORTS

N/A

8.2 REPORTING TIME

N/A
9 REPORTING TO THE HPA (LOCAL AND REGIONAL SERVICES AND CDSC CENTRE FOR INFECTIONS)

N/A

10 ACKNOWLEDGEMENTS AND CONTACTS

This National Standard Method was initiated and developed by the National Standard Methods Working Group for Clinical Virology (http://www.hpa-standardmethods.org.uk/wg_virology.asp). The contributions of many individuals in clinical virology laboratories and specialist organisations who have provided information and comment during the development of this document, and final editing by the Medical Editor are acknowledged.

The National Standard Methods are issued by Standards Unit, Department for Evaluations, Standards and Training, Centre for Infections, Health Protection Agency, London.

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Health Protection Agency
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London
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E-mail: standards@hpa.org.uk
APPENDIX 1: HAEMOLYTIC SYSTEM FOR CFT

A haemolytic system is prepared as follows:

- Wash SRBCs until the supernate is clear (usually 3 washes) in VBS. Resuspend washed SRBC to approximately 10% in VBS.
- Set up haematocrit to determine packed cell volume – centrifuge at 2500rpm for 20 minutes. Adjust concentration of SRBC suspension to 4%. If haematocrit reading is 12, make a 4% suspension by adding 4 mL SRBC to 8 mL VBS. This total amount could be altered as long as the proportions stay the same eg. 3+6 mL.
- Make up sufficient 4% SRBC for all the CFT plates (allow 2 mL of total haemolytic system per plate).
- Anti-sheep cell haemolysin (ASCH) is prediluted 1/10. Dilute ASCH further in VBS to give the dilution found in the C'/ASCH chessboard titration to give the same volume as the 4% SRBCs.
- Pour SRBC suspension into ASCH dilution. Mix well by pouring several times back and forth between the bottles. Test by adding equal volumes of each eg. 300 µL ASCH to 300 µL 3HD50 C’ and incubate for 10 minutes at 37°C. Store remainder at 4°C overnight until the next step of the test.

APPENDIX 2: VERONAL BUFFERED SALINE (VBS)

This is used throughout the test to maintain an optimum pH of 6.8 - 7.4 and to allow full fixation of complement at high dilutions of serum where, in saline, the calcium and magnesium concentrations would be sub-optimal. In this way greater differences of the titre between paired sera may be demonstrated.

It is prepared as a five-times concentrate. This concentrate is kept at 4°C. For use, dilute the concentrate 1/5 in deionised water ie 400 mL x 5 VBS +1600 mL H2O. Dispense to VBS bottles and store in the fridge at 4°C.

Stock VBS/AZIDE (0.2%)

Add 0.2 g of Sodium Azide to 100 mL of VBS. Ensure it is completely dissolved. Store at 4°C.

Working Strength VBS/AZIDE

Make 1:10 dilution of stock, 0.2% VBS AZIDE. For example, make up 100 mL, working strength VBS/AZIDE, by adding 10 mL of 0.2% stock to 90 mL of VBS. Store at 4°C.

Stock Sodium azide (0.2%)

Add 0.2 g of Sodium azide to 100 mL of deionised water. Ensure the Sodium azide is completely dissolved. Store at 4°C.

Working Strength Sodium azide

Make 1:10 dilution of stock, 0.2% Sodium azide. For example, make up 100 mL working strength by adding 10 mL of 0.2% stock to 90 mL of deionised water. Store at 4°C.
APPENDIX 3: TREATMENT OF ANTI-COMPLEMENTARY SERA

- Add 100 µL of patient’s serum to a Sarstedt vial. Add 25 µL of C’ (neat) incubate at +37°C for 30 minutes
- Make 1/16 dilution of the serum/C’ mixture in VBS as for routine sera. Inactivate at 56°C for 30 minutes
- Process specimen as normal in a CFT procedure

APPENDIX 4: TREATMENT FOR HAEMAGGLUTINATING SERA

- Label a Sarstedt vial with laboratory number
- Add 100 µL of patient’s sera
- Add 25 µL OF SRBC
- Incubate at 4°C for 1 hour
- Microfuge to sediment SRBC
- Make 1/16 dilution in VBS
- Inactivate at 56°C for 30 minutes
- Add to appropriate wells and process as normal
REFERENCES


