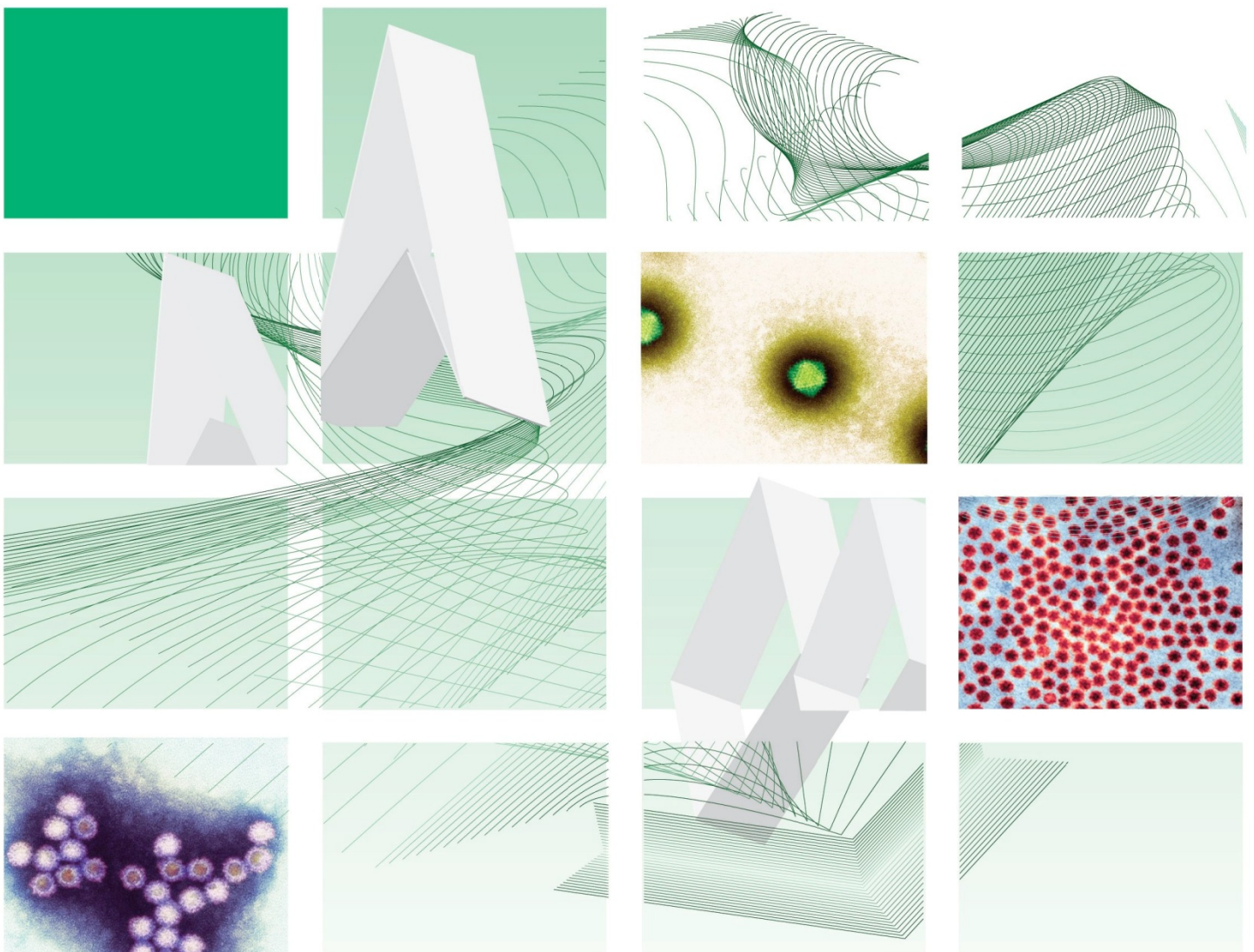




# UK Standards for Microbiology Investigations

## Complement Fixation Tests



## Acknowledgments

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UK Standards for Microbiology Investigations (SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website <http://www.hpa.org.uk/SMI/Partnerships>. SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see <http://www.hpa.org.uk/SMI/WorkingGroups>).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the Medical Editors for editing the medical content.

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UK Standards for Microbiology Investigations are produced in association with:



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NICE has accredited the process used by Public Health England to produce Standards for Microbiology Investigations. Accreditation is valid for 5 years from July 2011. More information on accreditation can be viewed at [www.nice.org.uk/accreditation](http://www.nice.org.uk/accreditation).

For full details on our accreditation visit: [www.nice.org.uk/accreditation](http://www.nice.org.uk/accreditation).

## Amendment Table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from [standards@phe.gov.uk](mailto:standards@phe.gov.uk).

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment No/Date.	6/09.10.13
Issue no. discarded.	3.1
Insert Issue no.	3.2
<b>Section(s) involved</b>	<b>Amendment</b>
Whole document.	<p>Document has been transferred to a new template to reflect the Health Protection Agency's transition to Public Health England.</p> <p>Front page has been redesigned.</p> <p>Status page has been renamed as Scope and Purpose and updated as appropriate.</p> <p>Professional body logos have been reviewed and updated.</p> <p>Standard safety references have been reviewed and updated.</p> <p>Scientific content remains unchanged.</p>

Amendment No/Date.	5/02.11.11
Issue no. discarded.	3
Insert Issue no.	3.1
<b>Section(s) involved</b>	<b>Amendment</b>
Whole document.	Document presented in a new format.
References.	Some references updated.

# UK Standards for Microbiology Investigations<sup>#</sup>: Scope and Purpose

---

## Users of SMIs

- SMIs are primarily intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK.
- SMIs provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests.
- SMIs provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

## Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages.

Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

## Equal Partnership Working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies.

The list of participating societies may be found at <http://www.hpa.org.uk/SMI/Partnerships>. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and Working Groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process.

SMIs are developed, reviewed and updated through a wide consultation process.

---

<sup>#</sup> Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

## Quality Assurance

NICE has accredited the process used by the SMI Working Groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008.

SMIs represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development.

The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

## Patient and Public Involvement

The SMI Working Groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

## Information Governance and Equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions.

The development of SMIs are subject to PHE Equality objectives [http://www.hpa.org.uk/webc/HPAwebFile/HPAweb\\_C/1317133470313](http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1317133470313). The SMI Working Groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

## Legal Statement

Whilst every care has been taken in the preparation of SMIs, PHE and any supporting organisation, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made to an SMI, it must be made clear where and by whom such changes have been made.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the time of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

SMIs are Crown copyright which should be acknowledged where appropriate.

### **Suggested Citation for this Document**

Public Health England. (2013). Complement Fixation Tests. UK Standards for Microbiology Investigations. V 18 Issue 3.2. <http://www.hpa.org.uk/SMI/pdf>.

## Scope of Document

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The SMI describes the procedure for performing complement fixation tests (CFT) on serum specimens<sup>1</sup>.

This SMI should be used in conjunction with other SMIs.

## 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<sup>2</sup>.

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.

## Technical Information/Limitations

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N/A



# 1 Safety Considerations<sup>3-19</sup>

---

## 1.1 Specimen Collection<sup>3,4</sup>

Appropriate hazard labelling according to local policy.

Specimens may contain blood borne viruses.

## 1.2 Specimen Transport and Storage<sup>3-8</sup>

Compliance with current postal and transportation regulations is essential.

## 1.3 Specimen Processing<sup>3-19</sup>

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 Type of Specimens

Serum

## 2.2 Optimal Time 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<sup>3,4</sup>

---

## 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 (25µL)
- 25µL “dropper” disposable pipettes
- Stepper pipettes (25µL)
- +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 12mm)
- Storage vials
- Robotic Microtitre plate processor (eg Kemble)
- 10mL and 1mL 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/Procedure<sup>3,4</sup>

### 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 7mL distilled water to 1mL of reconstituted complement.

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

Dilution	1/30	1/38	1/47	1/59	1/73	1/92	1/114	1/143	1/179	1/224
Distilled Water	3.5									
Reconstituted Complement	0.5									
VBS	Add 8mL	2	2	2	2	2	2	2	2	2
	Mix	Mix	Mix	Mix	Mix	Mix	Mix	Mix	Mix	Mix
	Transfer 8mL to next tube	Transfer 8mL to next tube	Transfer 8mL to next tube	Transfer 8mL to next tube	Transfer 8mL to next tube	Transfer 8mL to next tube	Transfer 8mL to next tube	Transfer 8mL to next tube	Transfer 8mL to next tube	Discard 8mL

**Figure 1:** Procedure for performing GPC' dilutions

Label a microtitre plate as illustrated in Fig. 2:

		1	2	3	4	5	6	7	8	9	10	11
<b>Haemolytic serum dilutions</b>		<b>Complement</b>										
		1/30	1/38	1/47	1/59	1/73	1/92	1/114	1/143	1/179	1/224	control
25	A											
50	B											
100	C											
200	D											
400	E											
800	F											
Control	G											

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

- To each well add 50µL (2 volumes) of VBS. Add 25µL (1 volume) of each dilution of GPC' to the appropriate column of wells. Add an additional volume (25µL) of VBS to column 11
- Gently mix, cover with a microtitre plate cover and place at +4°C overnight
- In labelled 75x12mm test tubes, prepare 1mL volumes of doubling dilutions of haemolysin in VBS from 1/25 to 1/800. Add 1mL of VBS to a tube labelled Control
- Prepare 4% SRBCs in VBS (see Appendix 1) and add 1mL to each of 7 labelled bijoux bottles
- Mix 1mL of the appropriate dilution of haemolysin (see Appendix 1) or control with the corresponding 1mL 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 25µL 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:

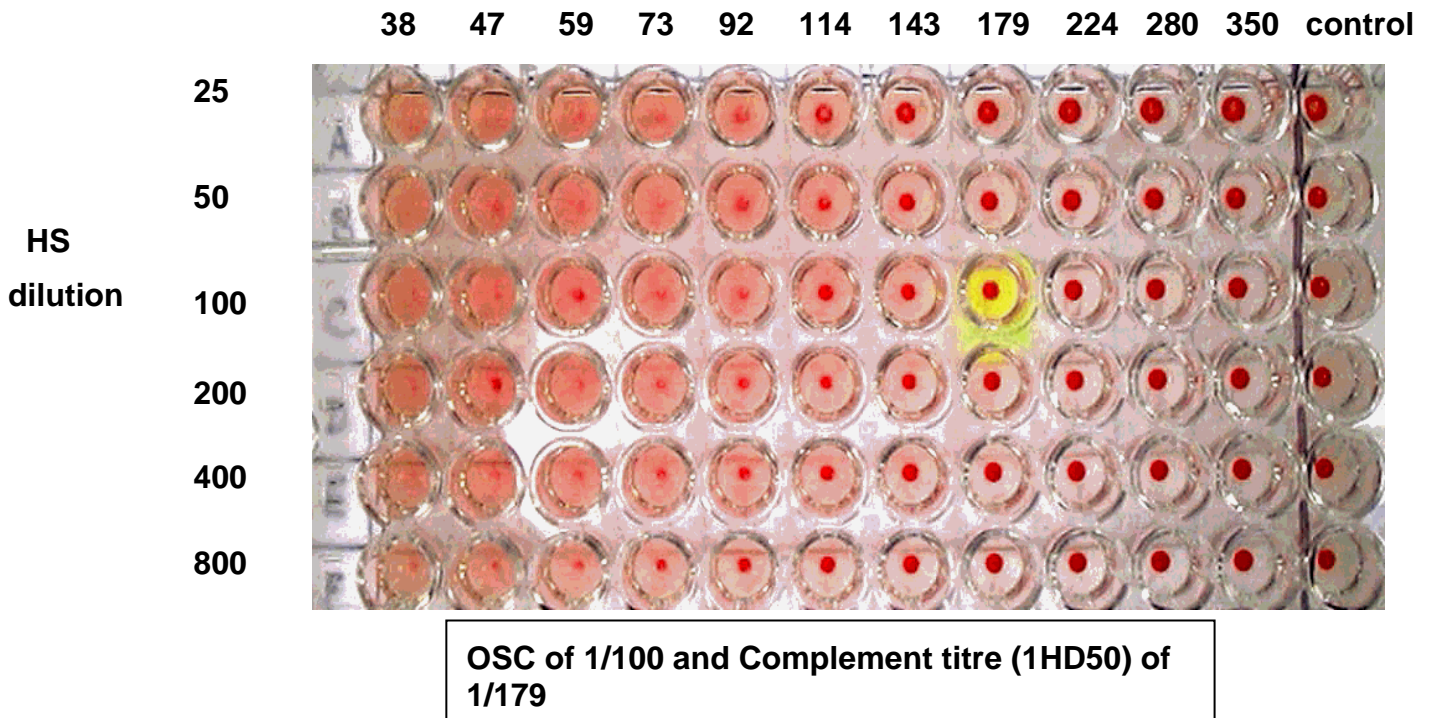
		1	2	3	4	5	6	7	8	9	10
	Haemolytic serum	Complement									
		1/38	1/47	1/59	1/73	1/92	1/114	1/143	1/179	1/224	control
A	1/25	0	0	Tr	1	2	3	4	4	4	4
B	1/50	0	0	0	Tr	1	2	4	4	4	4
C	1/100	0	0	0	0	Tr	1	2	4	4	4
D	1/200	0	0	0	Tr	1	2	4	4	4	4
E	1/400	0	0	Tr	1	2	3	4	4	4	4
F	1/800	0	Tr	1	2	3	4	4	4	4	4
G	Control	4	4	4	4	4	4	4	4	4	4

**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  $1/143 \times 3 = 1/47$ .

## Complement dilution



**Figure 4:** Photograph of expected haemolysin/GPC' chessboard titration

### 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 $\mu$ L 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 a 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

Antigen dilution		1	2	3	4	5	S/C	7	8	9	3HD50	1HD50	½ HD50
							6				10	11	12
10	A												
20	B												
30	C												
40	D												
80	E												
100	F												
	G												
	H												

**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 25µL VBS (column 6 is the serum control)
- To column 6, rows A-F, add a further 25µL VBS in place of the antigen
- To columns 1, 2 and 6, add 25µL of the appropriate antiserum. Double dilute from columns 2-5
- To columns 1-5, rows A-F, add 25µL of the appropriate antigen dilution
- Add 25µL 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 25µL VBS and appropriate antigen dilution (50µL in total) to columns 10-12, rows A-F. Tap the plate to mix the reactants
- To column 10, rows A-F, add 25µL 3HD50 GPC', to column 11 add 25µL 1HD50 GPC' and to column 12 add 25µL ½HD50 GPC'

Antigen dilution						S/C						S/C
	1	2	3	4	5	6	7	8	9	10	11	12
10	A											
20	B											
30	C											
40	D											
50	E											
60	F											
	G											
	H											
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 25µL 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 25µL 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 600µL 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						<u>S/C</u>	Antigen back titration			C' back titration			
		1	2	3	4	5		6	3HD50	1HD50	½HD50	3HD50	1HD50	½HD50
20	A													
30	B													
35	C													
40	D													
45	E													
50	F													
55	G													
60	H													

**Figure 7:** Template for setting up an antiserum/antigen titration

- Add 50µL of the appropriate antiserum dilution to column 1. Add 25µL VBS into columns 2-6, rows A-H
- Double dilute antiserum in each of rows A-H from columns 1-5. Add 25µL of the appropriate antiserum dilution to column 6. (Column 6 is the serum control)
- Add 25µL 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,  $\frac{1}{2}$  HD50 complement
- Antigen back titration - place 25 $\mu$ L of VBS into wells 7, 8 and 9 in row A. Place 25 $\mu$ L of antigen at its working dilution into each of the three wells. Place 25 $\mu$ L of 3HD50 complement into column 7, row A, 25 $\mu$ L of 1HD50 complement into column 8, row A and 25 $\mu$ L of  $\frac{1}{2}$ HD50 complement into column 9, row A
- C' back titration - place 50 $\mu$ L of VBS into wells 10,11 and 12 in row A. Place 25 $\mu$ L of 3HD50 complement into column 10, row A, 25 $\mu$ L of 1HD50 complement into column 11, row A and 25 $\mu$ L of  $\frac{1}{2}$ HD50 complement into column 12, row A

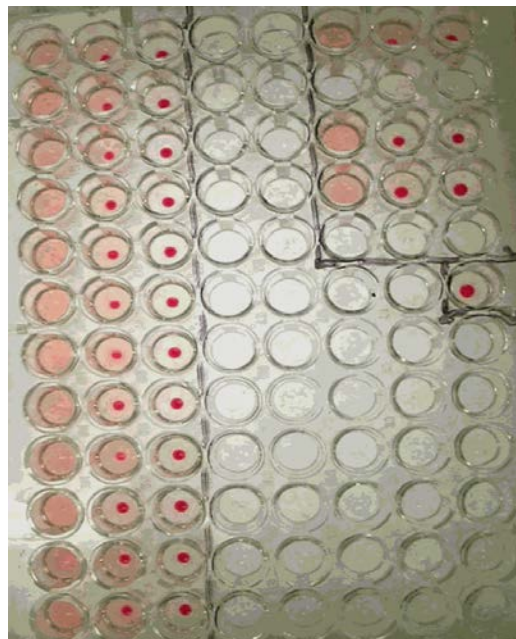
When the overnight incubation is complete, place plates at +37°C for 30 minutes. At the same time, incubate a sufficient volume of HS at +37°C (in a waterbath) for 30 minutes (see Appendix 1). Add 25 $\mu$ L of HS to all wells. Incubate at +37°C for 30 minutes and mix after 10, 20 and 30 minutes. Incubate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

Back titrations of complement of 3HD50, 1HD 50 and  $\frac{1}{2}$ HD50 in the presence of each antigen.  
The expected readings for each antigen are 0, 2, 4

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



**Cell  
control**

**Figure 8:** Photograph of antigen/C back titrations

## 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 50µL of serum to 750µL 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 25µL of inactivated serum to rows H,G and C (row C is the serum control). Add 25µL 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 25µL 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 25µL 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 25µL to all wells. Tap plate to mix the reactants. Cover plate and incubate overnight at +4°C

Antigen ↓	Specimen dilution →	1/16 H	1/32 G	1/64 F	1/128 E	1/256 D	S/C C	B	A
Influenza A	1								
Influenza B	2								
Adenovirus	3								
Chlamydia	4								
Q2(Coxiella)	5								
RSV	6								
Mycoplasma	7								
Measles	8								
VZ	9								
HSV	10								

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

Antigen		Antigen back titration			C' back titration		
		3HD50	1HD50	½HD50	3HD50	1HD50	½HD50
Influenza A	1	A	B	C	D	E	F
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

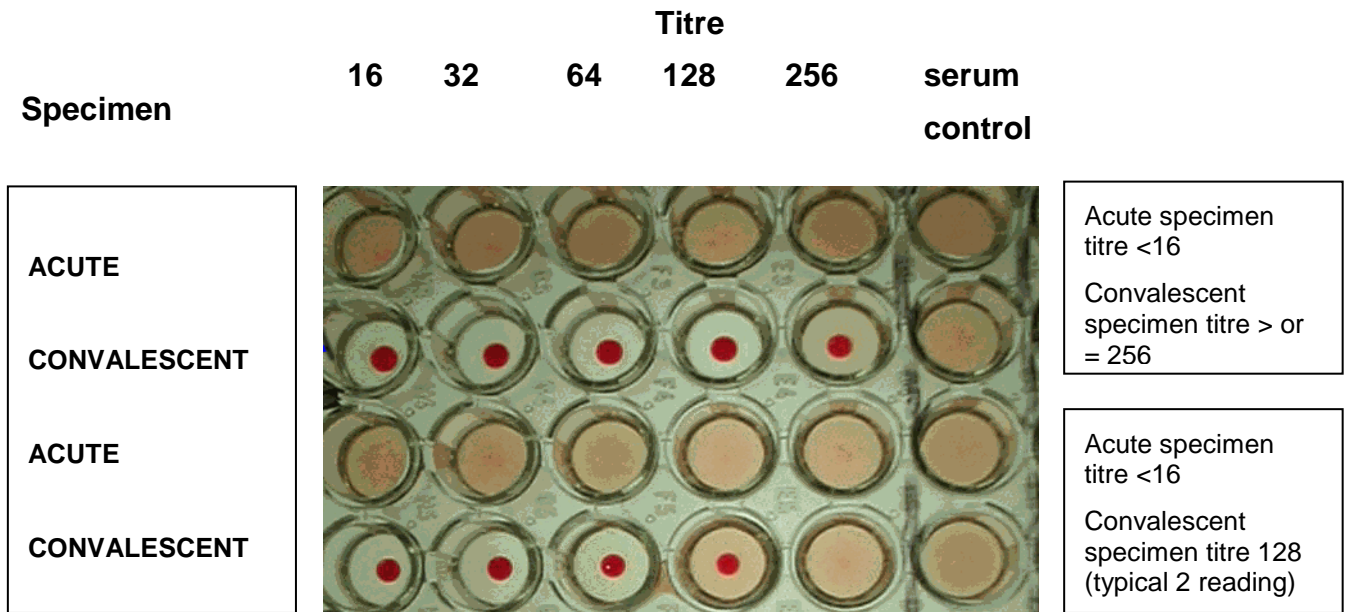
The CFT procedure is continued as follows:

- 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 25µL 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

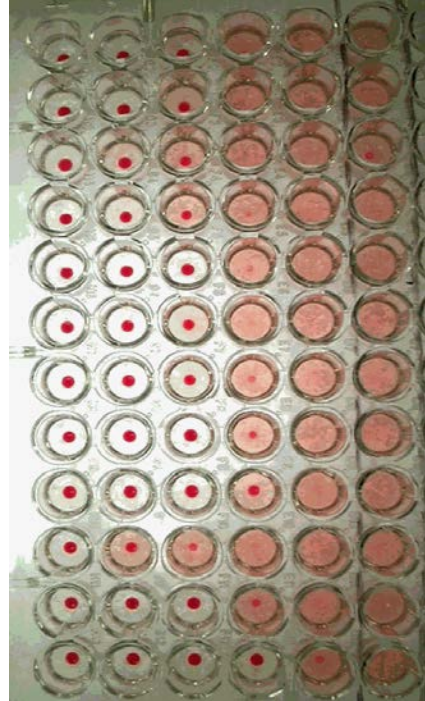


**Figure 11:** Photograph of acute and convalescent CFT

- 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 3<sup>rd</sup> well ie row F (see Fig. 12)

**Scoring of reactions in the CF test****0** = no. of cells remaining (complete lysis)**tr** = approx. 1 – 24% cells remaining**1** = approx. 25% cells remaining**2** = approx. 50% cells remaining**3** = approx. 75% cells remaining**4** = approx. 100% cells remaining

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



**Serum  
control**

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.

**Figure 12:** Photograph of antiserum control CFT

## 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<sup>20,21</sup>.

## 7 Limitations<sup>21</sup>

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 SMI is regularly reviewed it may not include emerging pathogens.

## 8 Reporting Procedure

### 8.1 Reports

N/A

## 8.2 Reporting Time

N/A

# 9 Notification to PHE<sup>22,23</sup> or Equivalent in the Devolved Administrations<sup>24-27</sup>

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The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days. For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health Protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

**Note:** The Health Protection Legislation Guidance (2010) includes reporting of HIV & STIs, HCAs and CJD under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

Other arrangements exist in Scotland<sup>24,25</sup>, Wales<sup>26</sup> and Northern Ireland<sup>27</sup>.



## Appendix 1: Haemolytic System for CFT

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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 4mL SRBC to 8mL VBS. This total amount could be altered as long as the proportions stay the same eg 3+6mL
- Make up sufficient 4% SRBC for all the CFT plates (allow 2mL 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 300µL ASCH to 300µL 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)

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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 400mL x 5 VBS +1600mL H<sub>2</sub>O. Dispense to VBS bottles and store in the fridge at 4°C.

### Stock VBS/AZIDE (0.2%)

Add 0.2g of Sodium azide to 100mL 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 100mL, working strength VBS/azide, by adding 10mL of 0.2% stock to 90mL of VBS. Store at 4°C.

### Stock Sodium azide (0.2%)

Add 0.2g of Sodium azide to 100mL 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 100mL working strength by adding 10mL of 0.2% stock to 90mL of deionised water. Store at 4°C.

### **Appendix 3: Treatment of anti-complementary sera**

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- Add 100µL of patient's serum to a Sarstedt vial. Add 25µL 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**

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- Label a Sarstedt vial with laboratory number
- Add 100µL of patient's sera
- Add 25µL 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

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1. Bradstreet CM, Taylor CE. Technique of complement fixation test applicable to the diagnosis of virus diseases. *Mon Bull Minist Health Public Health Lab Serv* 1962;21:96-104.
2. Schmidt NJ, Emmons RW. General principles of laboratory diagnostic methods for viral, rickettsial and chlamydial infections. In: Schmidt NJ, Emmons RW, editors. *Diagnostic Procedures for Viral Rickettsial and Chlamydial Infections*. 6th ed. Washington: American Public Health Association; 1989. p. 27-8.
3. European Parliament. UK Standards for Microbiology Investigations (SMIs) use the term "CE marked leak proof container" to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU *in vitro* Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: "The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes".
4. Official Journal of the European Communities. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices. 7-12-1998. p. 1-37.
5. Health and Safety Executive. Safe use of pneumatic air tube transport systems for pathology specimens. 9/99.
6. Department for transport. Transport of Infectious Substances, 2011 Revision 5. 2011.
7. World Health Organization. Guidance on regulations for the Transport of Infectious Substances 2013-2014. 2012.
8. Home Office. Anti-terrorism, Crime and Security Act. 2001 (as amended).
9. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Health and Safety Executive. 2013. p. 1-32
10. Advisory Committee on Dangerous Pathogens. Infections at work: Controlling the risks. Her Majesty's Stationery Office. 2003.
11. Advisory Committee on Dangerous Pathogens. Biological agents: Managing the risks in laboratories and healthcare premises. Health and Safety Executive. 2005.
12. Advisory Committee on Dangerous Pathogens. Biological Agents: Managing the Risks in Laboratories and Healthcare Premises. Appendix 1.2 Transport of Infectious Substances - Revision. Health and Safety Executive. 2008.
13. Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. *MMWR Surveill Summ* 2012;61:1-102.
14. Health and Safety Executive. Control of Substances Hazardous to Health Regulations. The Control of Substances Hazardous to Health Regulations 2002. 5th ed. HSE Books; 2002.
15. Health and Safety Executive. Five Steps to Risk Assessment: A Step by Step Guide to a Safer and Healthier Workplace. HSE Books. 2002.
16. Health and Safety Executive. A Guide to Risk Assessment Requirements: Common Provisions in Health and Safety Law. HSE Books. 2002.

17. Health Services Advisory Committee. Safe Working and the Prevention of Infection in Clinical Laboratories and Similar Facilities. HSE Books. 2003.
18. British Standards Institution (BSI). BS EN12469 - Biotechnology - performance criteria for microbiological safety cabinets. 2000.
19. British Standards Institution (BSI). BS 5726:2005 - Microbiological safety cabinets. Information to be supplied by the purchaser and to the vendor and to the installer, and siting and use of cabinets. Recommendations and guidance. 24-3-2005. p. 1-14
20. Curry A, Ashley CR. Quality assurance in electron microscopy. In: Snell JJS, Brown DFJ, Roberts C, editors. Quality Assurance Principles and Practice in the Microbiology Laboratory. London: Public Health Laboratory Service; 1999. p. 221-30.
21. Clinical Pathology Accreditation (UK) Ltd. Standards for the Medical Laboratory. Clinical Pathology Accreditation (UK) Ltd. Sheffield: 2004. p. 1-56.
22. Public Health England. Laboratory Reporting to Public Health England: A Guide for Diagnostic Laboratories. 2013. p. 1-37
23. Department of Health. Health Protection Legislation (England) Guidance. 2010. p. 1-112.
24. Scottish Government. Public Health (Scotland) Act. 2008 (as amended).
25. Scottish Government. Public Health etc. (Scotland) Act 2008. Implementation of Part 2: Notifiable Diseases, Organisms and Health Risk States. 2009.
26. The Welsh Assembly Government. Health Protection Legislation (Wales) Guidance. 2010.
27. Home Office. Public Health Act (Northern Ireland) 1967 Chapter 36. 1967 (as amended).