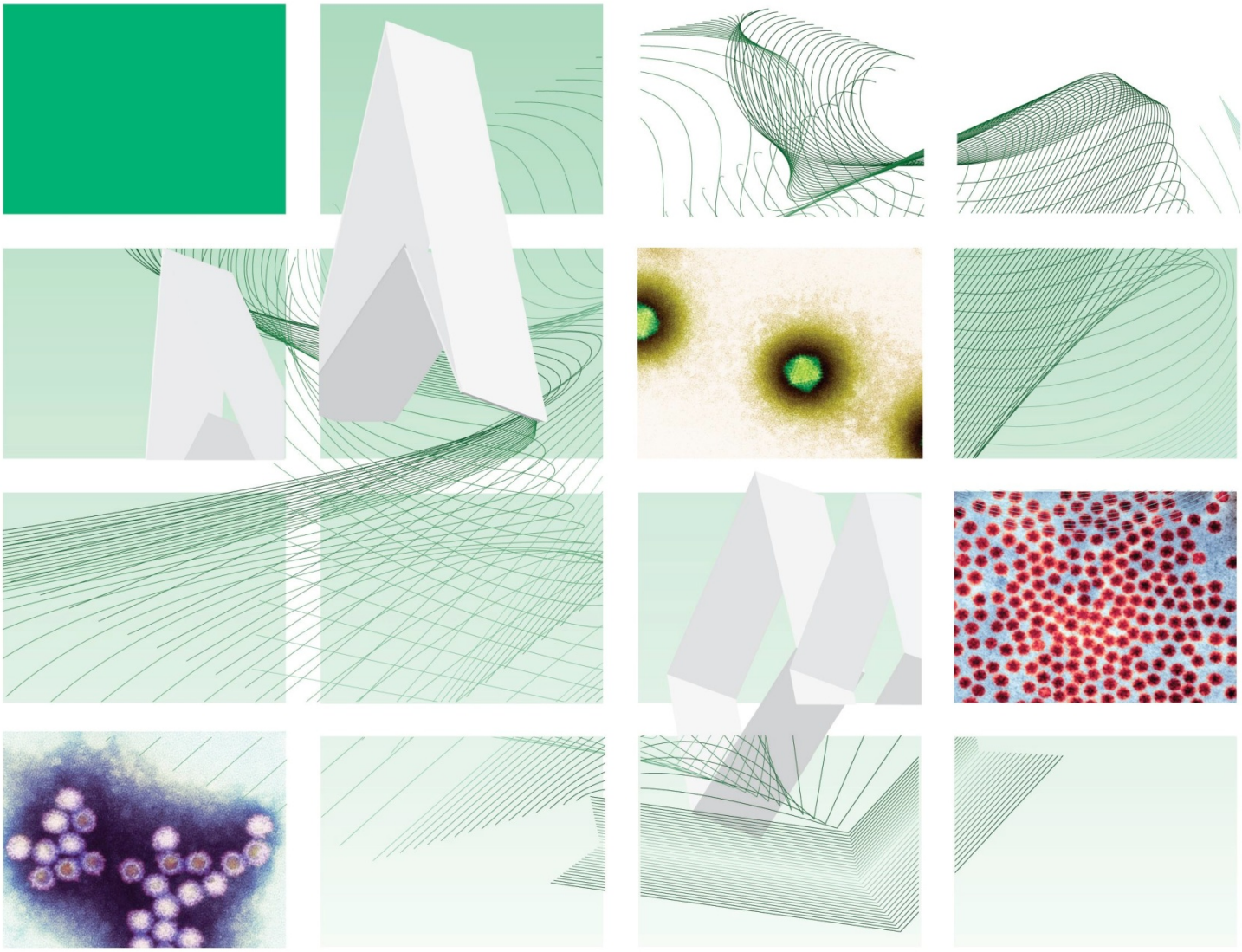
Public Health England

NHS

UK Standards for Microbiology Investigations

Immunofluorescence and Isolation of Viruses from Respiratory Samples



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Issued by the Standards Unit, Microbiology Services, PHE

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Acknowledgments

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:

Contents

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.

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

Amendment No/Date.	3/10.10.13
Issue no. discarded.	1.2
Insert Issue no.	1.3
Section(s) involved	**Amendment**
Whole document.	Document has been transferred to a new template to reflect the Health Protection Agency's transition to Public Health England. Front page has been redesigned. Status page has been renamed as Scope and Purpose and updated as appropriate. Professional body logos have been reviewed and updated. Standard safety references have been reviewed and updated. Scientific content remains unchanged.

Amendment No/Date.	2/20.04.12
Issue no. discarded.	1.1
Insert Issue no.	1.2
Section(s) involved	**Amendment**
Whole document.	Amendment to template.

UK Standards for Microbiology Investigations[#]: 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.

[#] 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. 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). Immunoflurescence and Isolation of Viruses from Respiratory Samples. UK Standards for Microbiology Investigations. V 22 Issue 1.3. http://www.hpa.org.uk/SMI/pdf.

Scope of Document

The SMI describes the detection and isolation of viruses from respiratory samples. Viral culture used to be considered the “gold standard” for the laboratory diagnosis of respiratory virus infections but is steadily being replaced by molecular methods. Information on making and maintaining cell cultures, molecular and serology methods are available from the Standards Unit website http://www.hpa.org.uk/SMI In spite of the increasing use of PCR for diagnosis the use of cell culture isolation methods for respiratory viruses will continue to be necessary to assess antigenic changes in respiratory viruses and allow phenotypic antiviral susceptibility testing.

This SMI should be used in conjunction with other SMIs.

Introduction

Background

Family Orthomyxoviridae

Genera *Influenzavirus A, Influenzavirus B, Influenzavirus C*

Influenza is a disease of global importance[1]. About 20% of children and 5% of adults worldwide develop symptomatic influenza A or B each year. There is also the chance of a pandemic occurring due to the ability of Influenza A to reassort. They cause a wide range of illnesses from symptomless infection through to various respiratory syndromes and disorders affecting the lungs, heart, brain, liver, kidneys and muscles to fulminate primary viral and secondary bacterial pneumonia[2]. The progression of the disease is affected by the patient’s age, the degree of pre-existing immunity, properties of the virus, smoking, co-morbidities, immunosuppresion and pregnancy. The virus is disseminated by coughing and sneezing with the dispersal of virus-laden respiratory droplets. Infection can also be acquired through fomites. Nasopharyngeal aspirate is considered the most sensitive specimen for the detection of influenza[3].

Influenza A, B, and C viruses are recovered best in roller cultures of PLC and Madin Darby dog kidney cells (MDCK). MDCK and other cells require a fortified medium containing trypsin for optimal sensitivity. Trypsin is needed to ensure the cleavage of virus HA into HA1 and HA2 which is necessary for virus replication. The virus replication is detected by haemadsorption (HA), haemagglutination or immunofluorescence.

Family Paramyxoviridae[4]

Subfamily *Paramyxovirinae*

Genus *Respirovirus*

Species *Human parainfluenza virus type 1, Human parainfluenzavirus type 3*

Genus *Rubulavirus*

Species *Human parainfluenzavirus type 2, Human parainfluenzavirus type 4, Human parainfluenzavirus type 4a, Mumps virus*

The parainfluenza and mumps viruses replicate well in roller cultures of NCI-H292 cells under a fortified medium containing trypsin for optimal sensitivity, and in PLC

without trypsin. The cytopathic effect (CPE) induced by these viruses may develop in 4 - 7 days but the cultures can be blind-passaged and held an additional week to ensure growth. The cells rarely become detached, and may not show obvious CPE. It is therefore crucial that these cell lines are haemadsorbed during the culture period and prior to discard to make sure that a virus is not overlooked. Details of haemadsorption are available in V 45 - Haemadsorption of Viruses.

Genus *Morbillivirus*

Species *Measles virus*

This is unlikely to be seen in a clinical laboratory as diagnosis is usually made on clinical presentation supported by serology. Respiratory samples are seldom submitted to the laboratory for virus culture but on occasions may be sent for direct immunofluorescence detection. Respiratory samples are tested by measles PCR. The virus is only shed early on in infection and therefore the window of opportunity for isolating this virus is very small. Virus isolation is also slow and is generally only carried out when susceptibility or epidemiological data is required. No one culture system has been determined to be optimal however the WHO recommends the use of Vero/SLAM cells[5]. For more details on measles see G 7 – Investigation of Red Rash.

Subfamily *Pneumovirinae*

Genus *Pneumovirus*

Species *Human Respiratory Syncytial Virus (RSV)*

Types *Human respiratory syncytial virus type A; Human respiratory syncytial virus type B*

RSV causes recurrent respiratory infections from infancy through adult life. Epidemics are common and may be hospital associated. Infection is spread by large particle droplets and by fomites, rather than by small particle aerosols. RSV may produce croup, bronchitis, bronchiolitis or interstitial pneumonia. It is the most common cause of respiratory disease in hospitalised infants who are younger than a year.

Culture is the best way to definitively identify this organism but is hard to achieve as the virus rapidly loses its viability. Samples should be stored at 4°C in transport medium and should be inoculated no later than four hours after the sample was taken. Roller Hep-2 cells are known to be sensitive to RSV[6]. Immunofluorescence is used in cases where rapid diagnosis is required.

Genus *Metapneumovirus*

Species *Human metapneumovirus*

Metapneumovirus infection is common in young children under 5 years of age. There is a broad spectrum of illness, similar to RSV, with fever, cough, and coryza common. The virus grows in LLCMK2, Hep 2 and MDCK but grows slowly and is more commonly diagnosed by PCR.

Family Picornaviridae

Genus *Enterovirus*

Species *Human enterovirus A-D*

Types *Coxsackievirus A (A21) and B (B4 B5), Echovirus, Enterovirus 68-71*

Enteroviruses are spread by aerosolised droplets, fomites and the faecal-oral route. These viruses produce CPE in NCI-H292, RMK, RD, trypsin-treated MA-104 and human diploid fibroblast cells, preferably in roller cultures[7].

Genus *Parechovirus*

Species *Human parechovirus*

Types *Human parechovirus types 1-3*

All three HPeV types have been isolated from individuals with respiratory symptoms. HPeV-1 is described as a cause of respiratory illness in children under 2 years of age. The virus grows in BGM cells. HPeV-3 has been associated with respiratory illness as well as gastroenteritis and rash, and can be grown in Vero cells.

Genus *Rhinoviruses*

Species *Human rhinovirus A, Human rhinovirus B*

Types *Rhinovirus (numbered types 1-100)*

Rhinoviruses are associated with upper respiratory tract infections or common colds which may be complicated by otitis media in children and sinusitis in adults[8]. Rhinovirus is also able to cause infections in the lower respiratory tract such as pneumonia, wheezing in children and aggravates conditions such as asthma and chronic obstructive pulmonary disease in adults. Human Rhinovirus has a relatively low optimum growth temperature of 33°C thought to be an evolutionary adaptation to the nasopharyngeal environment[8].

Rhinoviruses are spread by aerosols and fomites. These viruses produce CPE in NCI-H292, RMK, RD, trypsin-treated MA-104 and human diploid fibroblast cells, preferably in roller cultures at +33°C - 34°C.

Family Adenoviridae

Genus *Mastadenovirus*

Type *Adenovirus*

Adenoviruses are associated with diverse clinical syndromes. They are spread by droplets, fomites and the faecal-oral route. Adenovirus is a well known cause of respiratory illness in children and may produce upper or lower respiratory infection including bronchiolitis and pneumonia[9]. The virus is also connected with pharyngoconjunctival fever and epidemic conjunctivitis. These viruses are liable to become disseminated in patients who are immunocompromised, resulting in high mortality.

Most serotypes other than 40 and 41 replicate readily in any of the following human cell lines HEK, HEp2, HeLa, A549, HEL, and NC1-H292 cells with or without rolling[7]. The adenoviruses are among the easiest viruses to identify because they have a distinctive CPE and are unique in producing prodigious quantities of soluble antigens as they grow in cell culture, and these antigens possess many type and group specific properties that aid diagnosis.

Family Coronaviridae

Genus *Coronavirus*

Species *Human coronavirus 229E, human coronavirus OC-43, severe acute respiratory syndrome coronavirus*

Coronaviruses are common causes of colds. Coronavirus 229-E grows in HEL cells. However, it is generally hard to isolate and like coronavirus OC-43 may require organ culture. For this reason, culture of these organisms in a routine clinical laboratory is unlikely[7]. The SARS coronavirus caused severe lower respiratory tract disease in 2002-2003 in the Far East and in Canada, causing death in >7% of cases overall and in as many as 50% in those over 65 years. Cell culture is not usually undertaken in cases of suspected SARS; PCR and serological methods are used for laboratory diagnosis.

Technical Information/Limitations

N/A

1 Safety Considerations[10-26]

1.1 Specimen Collection[10,11]

Appropriate hazard labelling according to local policy.

1.2 Specimen Transport and Storage[10-15]

Compliance with current postal and courier transportation regulations is essential.

A suitable virus transport system must be used and the specimen should be placed in a sealed plastic bag, separately from the request form.

1.3 Specimen Processing[10-26]

N/A

2 Specimen Collection

2.1 Type of Specimens

Nasopharyngeal aspirates, nasal swabs, bronochoalveloar lavage, throat swabs/combined nose and throat swabs, sputum

2.2 Optimal Time of Specimen Collection

Respiratory specimens should be taken within three days of illness and no later than five days. All specimens should be taken before anti-viral chemotherapy is commenced.

2.3 Correct Specimen Type and Method of Collection

Duplicate specimens may be required for the exclusion of other microbial pathogens.

2.4 Adequate Quantity and Appropriate Number of Specimens

N/A

3 Specimen Transport and Storage[10,11]

3.1 Time between Specimen Collection and Processing

Specimens should be transported to the laboratory and processed as soon as possible. Specimens that may be delayed should be refrigerated prior to transportation to the laboratory.

3.2 Special Considerations to Minimise Deterioration

Samples should be refrigerated at 4°C if there is likely to be a delay in processing. If the delay is likely to exceed 24hr, the sample should be stored at -70°C and thawed prior to processing. Repeated freezing and thawing should be avoided.

4 Equipment and Reagents

4.1 Equipment

N/A

4.2 Reagents

N/A

5 Specimen Processing/Procedure[10,11]

5.1 Test Selection

Under circumstances where a more rapid result is required, electron microscopy (EM) or immunofluorescence (IF) or a commercial antigen detection assay may be used to provide an interim report but this result should be confirmed by culture where possible. In certain cases a validated PCR method may also be used.

IF is recommended as a rapid diagnostic test and also enables the quality of the sample taken to be determined. If there is a lack of epithelial cells on the slide it is unlikely that any virus will be detected. The chances of successful culture are also going to be reduced. In this case it is recommended that the doctors are asked to provide a repeat sample.

5.2 Culture and Investigation

5.2.1 Specimen processing

Nasopharyngeal aspirate

- A fine tip plastic pipette should be used to wash any mucoid material from the plastic tube (disposable mucus extractor) into 2mL of PBS in a plastic centrifuge tube. The sample should then be homogenised
- Approximately 500µL of the mixture should then be transferred into VTM for virus isolation
- It is recommended that a further 10mL of PBS is added to the plastic centrifuge tube and that the tube is then centrifuged at 380×*g* for 10 minutes in the centrifuge
- The supernatant should then be removed and re-suspended in 200µL of PBS
- Repeat above steps until all mucoid material has been removed. This aids clarification of the cells

Swabs

- Swabs should arrive broken off into VTM. Dry swabs or those in bacteriological transport medium are unacceptable, and repeat specimens in the correct format should be requested
- Ensure the container top is tight. Vortex mixture for 15 seconds to dislodge material on the swab into the transport medium

- Store the specimen at -80°C

5.2.2 Microscopy technique

Slides prepared for IF microscopy should preferably be prepared from material taken directly from the aspirate. When this is not possible a slide for examination may be prepared from the specimen if received in virus transport medium (VTM). Where the sample is inadequate, a repeat should be requested.

A slide should be labelled and 10 – 25µL of cell suspension, depending upon the density of the cell suspension, to 8 wells of the slide. The slide should then be dried on a Hot Plate. If insufficient material exists, then the number of wells can be reduced accordingly.

The slides should be fixed in acetone at room temperature for 10 minutes and then air dried.

This slide is now ready for immunofluorescent staining which should be carried out according to the manufacturer's instructions.

5.2.3 Isolation[7]

The purpose of virus isolation is to demonstrate the presence and viability of viruses in clinical specimens.

A variety of cell cultures are available. Traditionally all the viruses discussed in this SMI grow best in human tissue lines such as human embryonic lung tissues (HEL). Some laboratories include dog kidney cells (MDCK) in their cell line profile as parainfluenza and influenza viruses fail to grow in HEL cells.

The sample produced during basic specimen processing which should now be well mixed and clarified is inoculated into the selected cell culture monolayer(s). This work should be carried out in a class I safety cabinet. The tubes should be labelled so that the side containing the monolayer can be easily identified and the tubes should always be stored on that side. The inocula are adsorbed to the monolayer at ambient temperature. The length of time that these organisms have to absorb to the cell layer is crucial to sensitivity. Where possible, they should be left overnight. The cultures are then re-fed with maintenance medium and incubated at 35°C - 36°C for seven to fourteen days. HEL cell lines should be rolled in a roller drum or agitated on rocker platforms. The tubes should be read every 24 or 48 hours to check for the development of cytopathic effects and contamination under light microscopy.

Sub-passage of the cell cultures should not normally be required in order to produce the appearance of a CPE and should only be necessary if the cell cultures become contaminated with bacteria or fungi, or display degeneration of the cell sheet.

5.3 Identification

Within the laboratory

Identification of the isolates can be done in a number of ways:

- Cytopathic effect: see table in the appendix. Interpretation of CPEs can be very subjective and experience in reading cell cultures is very important. If in any doubt get a second opinion. CPEs should be confirmed using immunofluorescence, PCR or via a reference laboratory. It is possible for

misidentification and contamination to occur. Therefore any unusual findings should result in a request for a second sample where possible

- Immunofluorescence antigen detection is normally carried out to confirm the results of the CPE; this method does not detect all virus types and new virus strains or types will be missed. Follow the manufacturer's instructions
- Haemadsorption: this method can be found in V 45 – Haemadsorption of Viruses
- PCR either single or multiplex has proved to be very useful. Studies have shown that where patients are diagnosed on the basis of cell culture and immunofluorescence, the number of cases detected is underestimated

5.4 Referral to Reference Laboratories

- Isolates for antiviral susceptibility testing should be sent to the Antiviral Susceptibility Reference Laboratory, Public Health England
- Influenza isolates should be submitted to the WHO National Influenza Laboratory, Public Health England, Colindale for typing

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.

It is essential that laboratories have evidence of adequate validation of methods, equipment and commercial and in-house test procedures demonstrating that they are fit for purpose.

7 Limitations

Successful isolation of organisms depends on correct specimen collection, transport, storage and processing; the quality and range of cell lines used and the use of correct conditions for culture and the provision of adequate/suitable clinical information.

Only cell lines proven to be susceptible to respiratory viruses should be used and susceptibility should be checked on acquisition and at regular intervals. Cells retrieved from liquid nitrogen storage should be checked for sensitivity before use.

The procedure(s) in these documents aim to describe good microbiological standards 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 under regular review, it may not include emerging pathogens.

The above guidance should be supplemented with local assessments of limitations.

8 Reporting Procedure

8.1 Reports

Negative specimens should be reported as eg "Virus not isolated". "IF negative for…".

Positive specimens should be reported as eg “Influenza isolated”. “IF positive for…”.

If further tests are to be carried out, a report should be issued stating further results are pending.

9 Notification to PHE[27,28] or Equivalent in the Devolved Administrations[29-32]

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, HCAIs and CJD under ‘Notification Duties of Registered Medical Practitioners’: it is not noted under ‘Notification Duties of Diagnostic Laboratories’.

Other arrangements exist in Scotland[29,30], Wales[31] and Northern Ireland[32].

Influenza A, B, and C viruses: All should be reported with clinical and/or risk factor data desirable as part of the report.

Parainfluenza virus types 1, 2, 3, 4A, 4B: All should be reported.

Mumps virus: All should be reported with travel details, and clinical and/or risk factor data desirable as part of report.

Measles virus: All should be reported, with travel details, and clinical and/or risk factor data desirable as part of report.

Respiratory Syncytial Virus (RSV): All should be reported.

Rhinovirus: All should be reported.

Adenovirus: All should be reported.

Coronavirus*:* All should be reported and complete travel fields.

Appendix: CPE Appearances

CPE cause	Usual Growth Time (days)	Appearance in HEL	PLC-PRF5	MDCK
Influenza	3 – 5	No growth	Haemadsorbs	Haemadsorbs
Parainfluenza	3 - 5	No growth	Haemadsorbs	Haemadsorbs
RSV	5 - 6		Small rounding syncytia (cells tend to group and merge), some areas thin and clear	
Rhinovirus	4 - 10		Characteristic refractile rounding of cells. CPE identical to that produced by enteroviruses	
Adenovirus	2 - 10	'Ballooning' throughout, rounding elongated, slightly grainy, especially at the edge of the cell sheet		
Enteroviruses		Cells become rounded, refractile and ultimately shrink before detaching from the cell surface		

Pictures of CPE effects can be found on the CVN website http://www.clinicalvirology.org/

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