

NATIONAL STANDARD METHOD

RESPIRATORY VIRUSES

QSOP 60

Issued by Standards Unit, Evaluations and Standards Laboratory
Centre for Infections



UK Clinical Virology Network



Association of Medical Microbiologists
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RESPIRATORY VIRUSES

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Page no: 1 of 38

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RESPIRATORY VIRUSES

Issue no: 1 Issue date: 08.08.08 Issued by: Standards Unit, Evaluations and Standards Laboratory
QSOP 60i1

Page no: 2 of 38

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

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INDEX

STATUS OF NATIONAL STANDARD METHODS	2
INDEX	3
AMENDMENT PROCEDURE	5
INTRODUCTION	6
1 ADENOVIRUS	8
1.1 BASIC VIROLOGY AND TAXONOMY	8
1.2 LABORATORY DIAGNOSIS.....	8
1.3 CLINICAL SYMPTOMS	9
1.4 EPIDEMIOLOGY.....	10
1.5 PREVENTION AND CONTROL.....	11
2 CORONAVIRUSES	12
2.1 BASIC VIROLOGY AND TAXONOMY	12
2.2 EPIDEMIOLOGY.....	12
2.3 CLINICAL FINDINGS	13
2.4 LABORATORY DIAGNOSIS.....	13
2.5 TRANSMISSION.....	14
2.6 PREVENTION AND CONTROL	14
3 HUMAN BOCAVIRUS (HBOV)	15
3.1 BASIC VIROLOGY.....	15
3.2 LABORATORY DIAGNOSIS	15
3.3 CLINICAL SYMPTOMS	15
3.4 TRANSMISSION.....	15
3.5 EPIDEMIOLOGY.....	15
3.6 PREVENTION AND CONTROL	15
4 HUMAN METAPNEUMOVIRUS (HMPV)	15
4.1 BASIC VIROLOGY.....	15
4.2 LABORATORY DIAGNOSIS	15
4.3 CLINICAL SYMPTOMS	15
4.4 TRANSMISSION.....	16
4.5 EPIDEMIOLOGY.....	16
4.6 PREVENTION AND CONTROL	16
5 INFLUENZA VIRUS TYPES A AND B	16
5.1 BASIC VIROLOGY.....	16
5.2 LABORATORY DIAGNOSIS.....	17
5.3 CLINICAL SYMPTOMS	18
5.4 TRANSMISSION.....	19
5.5 EPIDEMIOLOGY.....	19
5.6 PREVENTION AND CONTROL	20
6 INFLUENZA VIRUS TYPE C	22
6.1 BASIC VIROLOGY AND TAXONOMY	22
6.2 LABORATORY DIAGNOSIS.....	22
6.3 CLINICAL SYMPTOMS	22
6.4 EPIDEMIOLOGY.....	22
6.5 ANTIVIRAL THERAPY	22

RESPIRATORY VIRUSES

Issue no: 1 Issue date: 08.08.08 Issued by: Standards Unit, Evaluations and Standards Laboratory Page no: 3 of 38
QSOP 60i1

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

Email: standards@hpa.org.uk

7	PARAINFLUENZA	22
7.1	BASIC VIROLOGY	22
7.2	LABORATORY DIAGNOSIS	23
7.3	CLINICAL SYMPTOMS	23
7.4	TRANSMISSION	23
7.5	EPIDEMIOLOGY	23
7.6	PREVENTION AND CONTROL	24
8	RHINOVIRUS	24
8.1	BASIC VIROLOGY	24
8.2	LABORATORY DIAGNOSIS	24
8.3	CLINICAL SYMPTOMS	24
8.4	TRANSMISSION	25
8.5	EPIDEMIOLOGY	25
8.6	PREVENTION AND CONTROL	25
9	RESPIRATORY SYNCYTIAL VIRUS	25
9.1	BASIC VIROLOGY	25
9.2	LABORATORY DIAGNOSIS	25
9.3	TRANSMISSION	26
9.4	EPIDEMIOLOGY	26
9.6	PREVENTION AND CONTROL	26
10	OTHER RESPIRATORY VIRUSES	27
10.1	HANTAVIRUS INFECTION	27
10.2	NIPAH VIRUS	27
11	RELEVANT NATIONAL STANDARD METHODS	28
12	ACKNOWLEDGEMENTS AND CONTACTS	29
	REFERENCES	30

RESPIRATORY VIRUSES

Issue no: 1 Issue date: 08.08.08 Issued by: Standards Unit, Evaluations and Standards Laboratory Page no: 4 of 38
 QSOP 60i1

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

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AMENDMENT PROCEDURE

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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.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment

RESPIRATORY VIRUSES

Issue no: 1 Issue date: 08.08.08 Issued by: Standards Unit, Evaluations and Standards Laboratory Page no: 5 of 38
QSOP 60i1

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

www.evaluations-standards.org.uk

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RESPIRATORY VIRUSES

Types of specimen:	Nasopharyngeal aspirates (NPA)	Nasal washouts
	Nose swab	Nasal secretions
	Throat swab	Sinus brushings/washings
	BAL	Respiratory secretions

INTRODUCTION

Respiratory virus infections occur commonly and are responsible for a great deal of morbidity worldwide. In the developed world respiratory viruses are responsible for a considerable amount of morbidity which has a significant economic impact. Mortality rates however are low. In contrast, in developing countries, viruses are responsible for approximately 20 to 30% of respiratory deaths in children². The spectrum of disease ranges from upper respiratory tract infections such as common colds to infections of the lower respiratory tract manifesting as bronchiolitis or pneumonia.

Types of samples

The sample of choice for diagnosing virus infections of the upper respiratory tract is a nasopharyngeal aspirate. If this is not available, nose and throat swabs (combined) are acceptable but are not acceptable for diagnosis using the immunofluorescence method (see below). Bronchoalveolar lavage (BAL) is the preferred sample for lower respiratory tract infections but is not commonly available. Sputum is an acceptable sample although this may present problems in processing due to the nature of the specimen which is frequently mucoid. Consequently if a cough is non-productive and not severe enough to warrant taking a BAL sample then a sample from the upper respiratory tract may be the only sample available. In all cases the quality of the sample is paramount in diagnosing respiratory virus infection. This is particularly true where immunofluorescence is used for rapid virus diagnosis (see below). Very often the nursing and medical staff collecting specimens are unaware of these vital facts and putting effort into educating these staff will pay dividends in the efficacy of respiratory virus diagnosis.

Diagnostic methods

In the 1960s diagnosis of respiratory virus infection relied on virus culture, either using a range of cell cultures or embryonated hen's eggs (in the case of Influenza viruses). In the 1970s a significant step forward was rapid virus diagnosis (eg the same day) by immunofluorescence, using cross-absorbed animal antisera. The utility of this technique was extended in the 1980s with the advent of monoclonal antibodies to viral epitopes³. Further advances are currently being made with the introduction of Polymerase Chain Reaction (PCR) techniques.

Diagnosing respiratory virus infection by growth in cell cultures is a sensitive method but is comparatively slow usually taking a few days, sometimes up to two weeks. Unsurprisingly this makes little or no contribution to the clinical management of the patient. A range of cell cultures is used in order to maximise the efficiency of diagnosis. This usually comprises of a continuous cell line (eg Hep2) and a semi-continuous, human diploid cell line (eg MRC5). Primary monkey kidney cells were used effectively for decades to grow influenza and parainfluenza viruses although in 2006 these were withdrawn and replaced with more ethically acceptable alternatives (eg Madin-Darby canine kidney cells, a continuous cell line).

Originally immunofluorescence, utilizing polyclonal virus specific antisera raised in animals (commonly rabbits), was restricted to a limited number of laboratories worldwide due to the complexity of making effective specific reagents. Considerable microscopy skills were required to reliably interpret the results. The replacement of polyclonal reagents with virus specific monoclonal antibody reagents solved the problem of limited supply with the former and made interpreting the results by microscopy easier. Immunofluorescence is a moderately sensitive technique and in experienced hands has a high degree of specificity. Furthermore a poor quality specimen containing very few respiratory epithelial cells can be identified and rejected as this technique is totally reliant on the cellular quality of the specimen.

RESPIRATORY VIRUSES

The strength and effectiveness of immunofluorescence was such that enzyme linked immunoassays (EIAs) never established themselves in routine laboratory diagnosis. They have however, in some hospitals, found a niche as rapid point of care screening tests at the patient's bedside, to diagnose either respiratory syncytial virus (RSV) or Influenza A virus. These tests compare reasonably well with the conventional tests of virus culture and immunofluorescence⁴. However it is essential for such point of care tests to be run in conjunction with routine testing in the virus laboratory so that the quality of the service can be ensured. Real-time PCR methods for respiratory virus diagnosis have recently started to be used and these are usually in a multiplexed format (ie testing for more than one virus, usually two or three at the same time)^{5,6}. Ideally the assays should include controls to check that the specimen does not include inhibitors that could give false negative PCR results. The introduction of these methods has allowed the routine diagnosis of viruses which are difficult or slow to detect by conventional techniques (eg coronaviruses, human metapneumovirus and rhinoviruses). The exquisite sensitivity of real-time PCR necessitates the use of caution in interpretation as viral nucleic acid may be detected for a number of weeks after initial infection. Running real-time PCR methods in parallel with conventional techniques, following their initial introduction, will aid interpretation as experience is gained.

RESPIRATORY VIRUSES

Issue no: 1 Issue date: 08.08.08 Issued by: Standards Unit, Evaluations and Standards Laboratory
QSOP 60i1

Page no: 7 of 38

This NSM should be used in conjunction with the series of other NSMs from the Health Protection Agency

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1 ADENOVIRUS

1.1 BASIC VIROLOGY AND TAXONOMY⁷

Adenovirus infections in humans were originally associated with respiratory disease⁸, however it was recognised that they could also be responsible for gastrointestinal and eye infections. Adenoviruses not only account for a significant proportion of viral respiratory infections in the general population, but they are also an important cause of morbidity and mortality in patients who are immunocompromised, particularly children, neonates and those undergoing bone marrow transplantation.

The family *Adenoviridae* comprises four genera, with viruses infecting humans belonging to the genus *Mastadenovirus*. There are six species (formerly called subgenera) called human adenovirus A to F, and sub-species B1 and B2. (A probable species G comprising adenovirus type 52 has recently been described⁹). The human adenoviruses are also allocated serotype numbers, based on neutralisation studies with animal antisera. There are at least 51 serotypes¹⁰, (see table 1), and there may be several genotypes within a single serotype.

Table 1

Species	Serotypes
A	12, 18, 31
B1	3, 7, 16, 21, 50
B2	11, 14, 24, 35
C	1, 2, 5, 6
D	8-10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, 42-49, 51
E	4
F	40, 41

Adenoviruses are non-enveloped with a diameter of around 70 - 90 nm and icosahedral symmetry. The capsid consists of 252 capsomers, 12 of which are pentons and 240 are hexons. Surface projections are often lost during preparation, or distinct filaments are seen to protrude from the 12 vertices. Hexons consist of a hexagonal base with a central cavity. Penton bases are tightly associated with one or two fibres; fibre proteins interact to form a shaft with a distal knob that interacts with the cellular receptor. The core consists of a nucleoprotein complex.

The genome comprises a single molecule of linear double-stranded DNA, of around 35 Kb.

Antigenic determinants that possess type-specific reactivity are found on the virion surfaces. The type-specific antigenic determinants are involved in antibody mediated neutralisation (hexons), or haemagglutination inhibition (fibres). Antigenic determinants that possess serogroup-specific reactivity are found on the hexons.

1.2 LABORATORY DIAGNOSIS

In the absence of an outbreak setting or other epidemiological clues, adenovirus infections are difficult to distinguish from similar syndromes caused by other pathogens. Several laboratory techniques are valuable in the diagnosis of infection with virus isolation, direct antigen detection, histopathology, electron microscopy, serology, and DNA detection all having utility in different settings.

1.2.1 TISSUE CULTURE

Adenoviruses can be isolated from stool, upper and lower respiratory tract secretions, conjunctival swabs, urine, CSF, blood, and biopsies of infected tissues. In common with many viral infections, collection of samples early in the illness allows optimum opportunity for recovery of virus, but viral excretion may occur for up to one week in respiratory illness and

RESPIRATORY VIRUSES

may be recoverable for up to two weeks from conjunctival swabs in pharyngoconjunctival fever.

1.2.2 HISTOPATHOLOGY

Lung infection is characterised by diffuse interstitial pneumonitis, necrosis of bronchial epithelium and mononuclear cell infiltrate. Virions are found in the nucleus, where they may form paracrystalline arrays seen as basophilic inclusions.

1.2.3 ELECTRON MICROSCOPY

Adenoviruses can be recognised by their unique morphology. The main use is in diagnosis of enteric infection.

1.2.4 DIRECT ANTIGEN DETECTION

Respiratory infection can be diagnosed by immunofluorescence using monoclonal antibodies against the hexon protein present in infected cells collected by nasopharyngeal aspiration, bronchoalveolar lavage, or in sputum. A result can be obtained within a couple of hours but it is less sensitive than tissue culture or PCR.

1.2.5 DNA DETECTION

Polymerase chain reaction amplification can have high diagnostic sensitivity and specificity, in addition to allowing a relatively rapid result in comparison to culture. These features make qualitative PCR an excellent means of detecting respiratory infections; however the high sensitivity may result in detection of virus nucleic acid for prolonged periods, the clinical relevance of which is yet to be determined.

Quantitative adenovirus PCR is becoming increasingly valuable in detecting and monitoring infection and effect of antiviral therapy in patients who are immunocompromised, particularly in the bone marrow transplant setting¹¹.

1.2.6 SEROLOGY

Specific IgM and IgG responses are generated by infection. IgM assays are not in common use, partly because detectable levels are not made in a significant proportion of cases. Diagnosis of infection can be made by IgG seroconversion, or a four fold or higher rise in antibody titre. Antibodies may be detected using complement fixation (CF), ELISA, IF, HAI, or neutralisation. CF is group specific whereas neutralisation and HAI are type specific. Serological diagnosis of infection is retrospective with respect to the acute illness, but may identify infections not diagnosed by virus detection.

1.3 CLINICAL SYMPTOMS

The outcome of infection is the result of interplay between host features, such as immunosuppression, and the viral serotype. A spectrum of outcomes is seen, from asymptomatic to severe pneumonia. The incubation period of endemic types is generally 4 to 10 days, and uncomplicated infection usually resolves within 1 week.

Apparent infection in childhood gives rise to the expected signs and symptoms of fever, runny nose, sore throat and cough; cervical adenitis and otitis media may also be present. The sore throat can be severe with exudative tonsillitis. When conjunctivitis occurs in addition to these features, the illness is called pharyngoconjunctival fever. The conjunctivitis often spreads to involve both eyes.

Bronchiolitis may be evident, with air trapping and peribronchiolar infiltrates and segmental or lobar atelectasis evident on the chest radiograph.

Severe pneumonia with fatality rates around 16% has been associated with types 3 and 7 infection in children^{12,13}. Bronchiolitis obliterans and residual lung damage has been observed as a complication¹⁴. Children under 2 years of age are most prone to severe infection. Extrapulmonary involvement (hepatitis, gastroenteritis, encephalitis) may occur in the immunocompetent host, often associated with the higher mortality rates observed.

RESPIRATORY VIRUSES

Respiratory infection in young adults is often with types 3, 4 and 7. Outbreaks of infections among military recruits were first described in the 1950s, typically involving types 4 and 7 but with types 3, 14, and 21 also occasionally implicated. High rates of hospitalisation and significant mortality occurred^{15,16}. Most recruits presented with fever, sore throat and persistent cough, progressing to signs and symptoms of pneumonia. Chest radiographs of cases presenting with pneumonia like illness may show patchy ground-glass infiltrates, mostly in the lower lung fields, although these features are not specific to adenovirus pneumonia. Progression of the disease may occur resulting in bilateral opaque lung fields. The epidemiology of respiratory adenovirus infections amongst military recruits is different to that in the rest of the population; however the reasons for this are not clear.

Adenovirus type 5 has been implicated in a pertussis-like syndrome, having been isolated in cases with and without evidence of *Bordetella pertussis* co-infection¹⁷.

Adenovirus infections in patients who are immunocompromised tend to be more severe than those in patients who are immunocompetent as well as more prolonged and challenging to treat. The case fatality rate for pneumonic infection can be as high as 60%¹⁸. Adenovirus infection is a particular problem in the bone marrow transplant setting, especially in children in whom an incidence of up to 31% has been found¹⁹. The associated mortality rates vary widely according to other factors, being higher if allogeneic transplant, T cell depleted, and young age group. Outcome is influenced by viral type also, with species C viruses often showing a more severe course of illness. Presentation of infection usually occurs in the first 3 months post-transplant and may be organ specific, causing isolated haemorrhagic cystitis, pneumonia, hepatitis, or colitis, or infection may be disseminated.

1.4 EPIDEMIOLOGY

Human adenoviruses are probably distributed worldwide. The virus is relatively resistant to physical and chemical agents, facilitating transmission by direct contact, water, contaminated objects, respiratory droplets and fomites. Their stability at low pH, such as that found in gastric secretions, allows faecal–oral spread.

Adenovirus infections may be sporadic, endemic and epidemic, occurring all year but with a higher frequency in winter and spring. Most infections occur in childhood, generating type specific immunity. Respiratory infections are usually caused by serotypes 1, 2, and 5 (species C) and types 3 and 7 (species B), although other types can be implicated (see table 2). Large outbreaks of upper and lower respiratory tract disease may occur in closed communities, notably in military barracks, commonly involving epidemic types 4, 7 and 21²⁰. Several respiratory syndromes are described for adenovirus infections, with significant crossover of causative serotypes (see table 2).

By age 10 years most of the population has had at least one adenovirus infection. In young children, between 2 and 7% of acute respiratory infections and up to 10% of respiratory infections leading to hospitalisation are caused by adenoviruses^{21,22} encompassing bronchitis, bronchiolitis, croup and pneumonia.

RESPIRATORY VIRUSES

Table 2

Syndrome	Classical Features	Associated Serotypes	
		Frequently	Less Frequent
Upper respiratory tract disease	Coryza, pharyngitis, fever, tonsillitis	1,2,3,5,7	4,6,11,18,21,29,31
Lower respiratory tract disease	Bronchitis, pneumonia, fever, cough	3,4,7,21	1,2,5,35
Pertussis-like syndrome	Paroxysmal cough, vomiting	5	1,2,3
Acute respiratory disease	Tracheobronchitis, fever, myalgia, coryza	4,7	3,14,21
Pharyngoconjunctival fever	Pharyngitis, conjunctivitis, fever	3,4,7	1,11,14,16,19,37

1.5 PREVENTION AND CONTROL

Respiratory adenovirus infection is spread via droplet, direct contact or indirect contact via a contaminated surface or object. Virus enters via the mucosal surfaces of the eye, nose or mouth. Standard respiratory infection control procedures, including isolation, hand-washing and disinfection, are indicated for patients with proven or suspected infection; in outbreak settings cohorting of patients may be necessary.

An oral enteric coated live serotype 4 and 7 vaccine has been effective in preventing outbreaks in military institutes^{15,23}, but has not been used in the wider population. The vaccine exploits immunity generated by local replication in the gut. More recently the vaccine has been unavailable, with re-emergence of adenovirus infections in military facilities²⁰.

1.5.1 ANTIVIRAL THERAPY

Upper respiratory tract infections generally resolve within one week and antiviral therapy is not required. Adenovirus pneumonia can be sufficiently severe, with possible long-term sequelae, to warrant antiviral therapy, however little data exists on the benefits of such treatment. Most information comes from the treatment of patients who are immunosuppressed, given either ribavirin or cidofovir in the context of severe or disseminated infection, rather than isolated respiratory tract disease²⁴. The results for ribavirin are mixed and difficult to interpret on a background of various patient populations. Some case reports suggest successful therapy with ribavirin^{25,26}, whereas some small patient series showed mixed results^{27,28}. *In-vitro* data has shown the activity of ribavirin to be adenovirus serotype and cell line dependent²⁹ potentially explaining the variations in its clinical efficacy. The main adverse effect of systemic ribavirin treatment is haemolytic anaemia.

Cidofovir has shown benefit in several settings. Cidofovir has delayed onset of adenovirus disease in an animal model setting, but could not fully suppress viral replication³⁰. There are several reports of successful cidofovir therapy for adenovirus infections of BMT or HSCT patients³¹⁻³³. Cidofovir has significant nephrotoxicity, often limiting its use. Neither ribavirin nor cidofovir is licensed for treatment of adenovirus infections.

In the setting of immunosuppression there is a correlation between survival of adenovirus infection and immunological recovery^{34,35}, indicating that antiviral therapy alone is unlikely to be sufficient for a successful outcome.

It seems likely that a system of early identification of infection in high risk groups, for example by quantitative PCR and pre-emptive therapy is most likely to be effective in disease management.

RESPIRATORY VIRUSES

Intravenous immunoglobulin has been used as part of a treatment protocol with variable results^{36,37}, but is not currently an established therapy.

Donor lymphocyte infusion has shown benefit in management of severe adenovirus infections in bone marrow transplant recipients³⁸. These adenovirus specific cytotoxic T cells have potential for a broad therapeutic effect but they are technically challenging to produce and have to be generated for each individual patient.

2 CORONAVIRUSES

2.1 BASIC VIROLOGY AND TAXONOMY

The coronaviruses are an important group of pathogens classified in the family *Coronaviridae* in the order *Niriviridales*. Within this virus family are two genera, *Coronavirus* and *Torovirus*. The coronaviruses are enveloped spherical viruses approximately 80-150 nm diameter. The envelope is studded evenly with club-shaped peplomers, each 12-20 nm which give a fringed corona-like appearance on electron microscopy. The capsid has helical symmetry, and contains a 27-31kb RNA genome which is single-stranded positive sense. The genus *Coronavirus* is divided into three groups. Of the human coronaviruses coronavirus 229-E (HCoV-229E), and coronavirus NL63 (HCoV-NL63), are in Group 1, while coronavirus OC43 (HCoV-OC43), human enteric coronavirus, coronavirus HKU1(HCoV-HKU1) and severe acute respiratory syndrome (SARS) –coronavirus (HCoV-SARS) are included in Group 2.

2.2 EPIDEMIOLOGY

A number of human coronaviruses are recognized as causing respiratory illness, with the prevalence of HCoV-NL-63, HCoV-OC43, HCoV-229E and HCoV-HKU1 varying across different centres³⁹⁻⁴¹. The spectrum of coronavirus infection ranges from mild coryza to severe life-threatening pneumonitis. Coronaviruses are second to rhinoviruses as causes of the common cold⁴². The first coronaviruses isolated (B814, 229-E and OC43) were isolated in human embryonic trachea organ cultures and were found to resemble the well described avian coronavirus infectious bronchitis virus⁴³. Both HCoV-OC43 and HCoV-229E have also been associated with exacerbations of wheeze in asthmatic children⁴⁴.

Coronavirus 229E

This virus was isolated in organ culture in the mid-1960s and was shown to cause colds in volunteer studies. HCoV-229E is a group I coronavirus. Seroepidemiological studies showed infection to be largely associated with undifferentiated respiratory illness, with a seasonal winter-spring pattern. Reinfections in the face of pre-existing antibody proved to be common⁴⁵. HCoV-229E remains a significant cause of colds all over the world.

Coronavirus NL63

This agent was discovered in the Netherlands in 2004⁴⁶ by using molecular techniques (VIDISCA) on supernatant from tissue culture inoculated with a nasopharyngeal aspirate from an infant with bronchiolitis. Other respiratory viruses described soon after, HCoV-NL and the New Haven coronavirus HuCo-NH, proved to be the same as HCoV-NL63. This group I coronavirus is found worldwide causing upper and lower respiratory illness. It is especially prevalent in the winter. Infections are seen at all ages, but are most common in those under 5 years of age. Coinfections with other respiratory viruses are frequent with HCoV-NL63 infection, particularly in hospitalized patients⁴⁷. HCoV-NL63 is common in croup⁴⁸ and is also frequent in the immunocompromised and those with underlying pulmonary disorders. The association of NL63 coronavirus and Kawasaki disease reported by the New Haven group has not been confirmed by later studies⁴⁹.

RESPIRATORY VIRUSES

Coronavirus HKU1

Coronavirus HKU1 is a group 2 coronavirus first found in Hong Kong in two patients with pneumonia⁵⁰. This virus is found worldwide in upper and lower respiratory tract infection in children, especially in those under 6 months⁵¹.

Coronavirus OC43

Like HCoV-229E the HCoV-OC43 causes community epidemics of colds in winter-spring, although less regularly than HCoV-229E⁵². The capacity of HCoV-OC43 for spread in closed communities has been demonstrated recently in outbreaks of influenza-like illness in elderly care homes⁵³.

SARS-CoV

In late 2002 an outbreak of a severe respiratory disease appeared in Guangdong Province in southern China. This disease, severe acute respiratory syndrome, had a high mortality rate and ultimately appeared in some 25 countries, killing some 774 individuals of over 8000 infected, before termination of the disease in 2005. The causative agent was shown to be a novel coronavirus⁵⁴⁻⁵⁶. The natural reservoir for similar viruses is the horseshoe bat, so it seems likely that SARS came originally from this source with possible amplification through caged palm civets in live animal markets in China⁵⁷ and later adaptation to human to human spread.

2.3 CLINICAL FINDINGS

Coronaviruses are associated with the spectrum of respiratory illness from colds to severe lower respiratory tract syndromes. Coronavirus 229-E and HCoV-OC43 are particularly associated with coryzal symptoms. Coronaviruses are being increasingly found in immunocompromised patients but there is little information on risk of progression to pneumonia. Coronavirus NL63 may be particularly associated with croup⁴⁸. The coronaviruses seem to have a limited role in causing exacerbations of asthma in children.

For upper respiratory tract infection with coronaviruses the incubation period is typically 2 days, producing an illness indistinguishable from a rhinovirus cold; virus is shed for about 5 days⁵².

For SARS, the incubation period was about 6 days (range 2 to 10) and hospitalization followed 3-5 days after the onset of the clinical symptoms. The initial presentation was fever, rigors, myalgia, malaise, dry cough, headache and dyspnoea⁵⁸. Watery diarrhoea was a feature of the Amoy Gardens outbreak in Hong Kong, probably associated with aerosol spread of virus via sewage⁵⁸. After an initial improvement there may be recurrence of fever, worsening lung function and progressing chest X-ray changes. Most improve with therapy with steroids and ribavirin but 20-36% need intensive care management and 13-26% progress to acute respiratory distress syndrome⁵⁸. Likelihood of death is age-related, highest in those over 60 years. In children the infection is less severe, and although the course is often protracted in teenagers, deaths are rare.

2.4 LABORATORY DIAGNOSIS

Isolation of many human respiratory coronaviruses is difficult and is not widely used. The best system is tracheal organ culture, which is not appropriate in the diagnostic laboratory setting. Coronavirus isolation in commonly employed cell cultures is of poor sensitivity other than HCoV-SARS. HCoV-229E may grow in fibroblasts such as MRC5 and in L132. HCoV-OC43 grows well in HRT18 (a human rectal tumour line)⁵⁹. The HCoV-SARS grows well in Vero cells giving a cytopathic effect at around 7 days⁵⁴. The virus can be identified as a coronavirus by electron microscopy from the cell culture material or directly from respiratory secretions^{54,55}.

Monoclonal antibodies have been developed for use in specific detection of HCoV-229E and HCoV-OC43 infection by immunofluorescence and immunoperoxidase techniques⁵⁹.

A range of serological techniques has been used for diagnosis of 229-E and OC-43 infection, including complement fixation tests, immunofluorescence, and neutralization. Rising titres

RESPIRATORY VIRUSES

detected by ELISA antibody testing correlated well with degree of cold symptoms in 229-E infection; development of neutralizing antibodies was less sensitive⁶⁰. However serology is not widely available for the coronaviruses associated with the common cold.

Antibody testing was used during the SARS epidemic as a diagnostic tool and has been regarded as the gold standard test. The Western blot assay using a sequence from the nucleocapsid protein had a 98% specificity for HCoV-SARS and a 90% sensitivity, 89% for IgG antibodies and 57% for IgM antibody⁶¹. Results using the immunofluorescent antibody test have been reported to have higher sensitivity and specificity⁶². Testing for neutralizing antibodies and for IgG and IgM antibodies by ELISA are also advocated⁶³ both on acute phase and convalescent phase (>21 days) sera⁶⁴.

During the SARS epidemic direct detection of HCoV-SARS was employed using a specific ELISA for detection of a nucleocapsid antigen⁶⁵ in nasopharyngeal material. This assay had good specificity (over 96%) but limited sensitivity compared with PCR, 52% in nasopharyngeal aspirate (NPA), 5% in urine, 55% in faeces. Presence of antigen was longlived – 83% of NPA samples positive at days 11 to 15 of illness and in 100% of faeces samples between days 21 and 32.

Because of the limited availability of serological testing and the limited efficacy of cell culture for many coronaviruses as well as the containment requirements for HCoV-SARS nucleic acid amplification methods are widely used for respiratory coronavirus diagnosis. Pancoronavirus primer sets have been described which claim to detect all coronaviruses^{39,66}. However the genetic variability among HCoV-OC43, HCoV-229E, and HCoV-NL63 is such that as many as 30% of coronavirus infections are not detected using such pancoronavirus methods^{67,68}. Specific primers for each coronavirus should ideally be used to maximize the diagnostic yield, with confirmation employing a second set of primers⁶⁸. Primers for diagnosis of HCoV-SARS have been made available on the WHO website⁶⁹. In the cases seen in Canada HCoV-SARS PCR detected just 71% of cases, 32% in nasopharyngeal swabs, 63% in faeces, and 59% from lower respiratory tract⁷⁰, demonstrating the need for additional serological testing in suspected cases. The use of real-time Taqman PCR on blood samples early in the illness offers reasonable sensitivity in a closed system^{58,71}. Diagnosis of SARS in England is summarized by the HPA at http://www.hpa.org.uk/infections/topics_az/SARS/micro.htm

2.5 TRANSMISSION

For coronaviruses droplet and close contact are the likely major routes of transmission. The OC43 and 229E viruses can survive several hours dried on surfaces⁷².

For SARS Co-V droplet and contact transmission appear to be most important, although some instances in the outbreak suggested possible airborne and fomites transmission⁷³. Transmission by contaminated hands touching mucous membranes of nose and eye is probably the major route of infection for the coronaviruses⁷⁴. The Amoy Gardens outbreak in Hong Kong suggested airborne transmission of HCoV-SARS spread by aerosols generated by flushing toilets in a high rise apartment block. The presence of HCoV-SARS in faeces⁷⁵ also suggests the possibility of faecal-oral transmission.

2.6 PREVENTION AND CONTROL

Infection-control measures for SARS-CoV should prevent airborne, droplet, and contact transmission⁷³. Potentially contaminated surfaces should be wiped regularly.

No commercial vaccines are licensed for coronavirus prevention. Inactivated SARS-CoV induces neutralizing antibodies after about two weeks⁷⁶.

There is little experience of the use of antivirals for coronaviruses other than SARS-CoV. In the SARS epidemic ribavirin was widely used but evidence of efficacy is unclear although it does inhibit SARS-CoV replication *in vitro*⁷⁷. Protease inhibitors including lopinavir-ritonavir and nelfinavir inhibit SARS Co-V *in vitro*. There was apparent benefit from protease inhibitor use in SARS cases⁷⁸. Use of steroids and interferon may also be of benefit in SARS although evidence is hard to evaluate⁷⁹.

RESPIRATORY VIRUSES

3 HUMAN BOCAVIRUS (HBOV)

3.1 BASIC VIROLOGY

Human Bocavirus (HBoV) was first described in September 2005; it was discovered by random polymerase chain reaction (PCR) cloning technique performed on pooled respiratory samples, followed by bio informatics analysis of the sequences of the resulting clones⁸⁰. This virus has currently been classified as belonging to the *Bocavirus* genus in the family *Parvoviridae*. So far there have been 2 closely related genotypes confirmed. Our knowledge regarding its occurrence, clinical profile and role as a causative agent of respiratory disease is still not clear.

3.2 LABORATORY DIAGNOSIS

Laboratory diagnosis is by nucleic acid detection eg PCR on respiratory secretions but is not in widespread use currently.

3.3 CLINICAL SYMPTOMS

HBoV is thought to be a common but under diagnosed cause of community – acquired respiratory infection in mainly children <3 years. It has been most frequently associated with children presenting with acute lower respiratory tract infection⁸¹.

3.4 TRANSMISSION

Spread of HBoV is presumed to be airborne and probably also by fomites.

3.5 EPIDEMIOLOGY

Studies so far show that the virus seems to be seasonal, being primarily found during the period autumn to spring. In published studies so far the virus was detected in 1.5%-11.3% of individuals with acute respiratory illness in North America, Europe, Asia and Australia suggesting global distribution⁸¹.

HBoV appears to differ in at least one aspect from infections with other respiratory viruses; their frequency of coinfection. Many studies have noted this with the variation in frequency being between 18% to 56 %⁸¹.

3.6 PREVENTION AND CONTROL

There is no vaccine. Airborne precautions and hand washing should be used to prevent spread.

4 HUMAN METAPNEUMOVIRUS (HMPV)

4.1 BASIC VIROLOGY

HMpV is not a new virus, but was discovered in 2001⁸². It is in the family *Paramyxoviridae*, subfamily *Pneumovirinae*. Pneumovirinae contain two genera, *Pneumovirus* (which contains the species RSV) and *Metapneumovirus* (which contains HMpV and avian pneumovirus which is genetically similar to HMpV)⁸³. There are two genogroups of HMpV, A and B.

4.2 LABORATORY DIAGNOSIS

Laboratory diagnosis is mainly by nucleic acid detection eg PCR on respiratory secretions. Culture is difficult and inappropriate for routine diagnosis. Commercial antigen detection tests are in development. IF is also a useful test⁸⁴.

4.3 CLINICAL SYMPTOMS^{85,86}

HMpV is a common but under-diagnosed cause of community-acquired respiratory illness in infants, children and adults⁸⁷. After an estimated incubation period of 5 - 6 days it causes upper and lower respiratory tract infections (URTI, LRTI), with symptomatology ranging from subclinical to severe pneumonitis, with most severe illness in the “normal” host when first infected.

RESPIRATORY VIRUSES

In infants/children under 2 years, HMpV is an important cause of bronchiolitis (RSV commonest) and pneumonia. An individual patient with HMpV is clinically indistinguishable from one with RSV and so clinical diagnosis is unreliable.

At other ages, the main illnesses caused are found in patients who are immunocompromised⁸⁸. Lower respiratory tract infections are more likely in patients who are non-immunocompromised older children/healthy adults: usually non-febrile URTI, with more severe illness in frail elderly or the chronically ill.

HMpV may have a role in the exacerbation of COPD in adults⁸⁹ and exacerbation of asthma in children. It is postulated that co-infection with RSV causes more severe illness than either infection alone. Management is supportive.

4.4 TRANSMISSION

Spread of HMpV is presumed to be airborne and probably also by fomites.

4.5 EPIDEMIOLOGY

Almost everyone has been infected by 5 years of age, with re-infections occurring throughout life. Like RSV, winter epidemics occur and it is a cause of healthcare associated infection. It is thought that illness is more severe with genogroup A. It is not known if there is cross-protection between genogroups A and B.

4.6 PREVENTION AND CONTROL

There is no vaccine. Airborne precautions and hand washing should be used to prevent spread.

5 INFLUENZA VIRUS TYPES A AND B

5.1 BASIC VIROLOGY

Influenza is an important infection in humans and animals. In humans influenza A has caused global pandemics, resulting in millions of deaths. Influenza A and B have caused regional and widespread epidemics and influenza C is associated with mild illness in sporadic cases and minor localised outbreaks.

Influenza viruses are classified in the genus *Orthomyxovirus*, in the family *Orthomyxoviridae* and they are negative sense RNA viruses.

When viewed under the electron microscope influenza virions are irregularly shaped spherical particles, between 80-120nm in diameter. They may also appear as filamentous or elongated structures.

Influenza viruses are enveloped, the lipid bilayer being derived from the host cell plasma membrane. Studded within the bilayer are two integral membrane proteins, Haemagglutinin (HA) and Neuraminidase (NA) in a ratio of about 1:5 HA to NA.

The lipid bilayer surrounds the matrix protein (M) which in turn surrounds a helical nucleocapsid which comprises nucleoprotein, RNA polymerase and eight segments of negative-sense RNA. The viral RNA codes for eight structural and two non structural proteins:

- The HA gene. Haemagglutinin, so called because it has the ability to agglutinate red blood cells, is essential for the virus to bind to host cell receptors, by the sialic acid receptors. In order for the virus to be infectious the HA must undergo cleavage into two receptors, HA1 and HA2
- The NA gene. Neuraminidase cleaves sialic acid residues on newly formed virions and is essential for release and spread of progeny virions. This is an important target for antiviral agents

RESPIRATORY VIRUSES

- The NP gene encodes for the nucleoprotein (NP), which forms a helix in association with the single stranded RNA genome
- The M gene codes for the two matrix proteins (M1, M2) by using different reading frames. The M1 protein stabilizes the virus whilst the M2 protein acts as an ion channel, facilitating the release of engulfed viral particles from host cell endosomes, thereby ensuring the release of RNA and the initiation of infection
- The NS gene codes for two non-structural proteins (NS1, NS2) again by using different reading frames
- The final sections of the RNA molecule code for the 3 subunits of RNA polymerase (PA, PB1, PB2). These are essential for the transcription and replication of virion RNA
- Three types of human influenza virus are recognised; types A, B and C. These types are distinguished by antigenic differences in their nucleoprotein and matrix protein. Further subtyping is based on the antigenicity of the two surface glycoproteins, Haemagglutinin and Neuraminidase⁹⁰.
- Immunity is induced by infection, or vaccination, with the production of neutralising antibodies. The influenza virus has a unique structure that allows it to undergo antigenic variation; this occurs in two forms: 'antigenic shift' and 'antigenic drift'
- Antigenic drift is a subtle process and involves the accumulation of uncorrected mutations within the genome resulting in changes to the antibody binding sites of HA, NA or both. Consequently a virus cannot be inhibited by antibodies against a previous strain, therefore allowing it to continue to spread in a partially immune population
- Antigenic shift is a more dramatic change in antigenicity and is only seen in influenza A. Antigenic shift results in the replacement of the HA, and sometimes NA, with novel subtypes which have not been present in human viruses for a long time, if ever. The source of these new genes is the large reservoir of influenza viruses seen in waterfowl. To date 9 HA and NA subtypes have been identified^{91,92}. It is antigenic shift that gives influenza A the ability to cause pandemics

5.1.1 NOMENCLATURE OF INFLUENZA VIRUSES

This was revised in 1980 by the World Health Organisation (WHO). Influenza viruses are named to include type, host of origin (unless human), geographical origin, strain number, year of isolation and the antigenic description of the HA and NA, the latter given in parentheses, eg A/chicken/Pennsylvania/1370/83 (H5N2) or, if it is found in a human, A/Shanghai/16/89(H3N2).

5.2 LABORATORY DIAGNOSIS

Within the diagnostic laboratory there are a number of methods used to diagnose influenza infection. These can be divided into those which look directly for the virus (antigen detection), and those which detect antibody to the virus (antibody studies).

5.2.1 ANTIGEN DETECTION

5.2.1.1 Immunofluorescence

Influenza virus can be detected rapidly in nasopharyngeal aspirate (NPA) and bronchio-alveolar lavage (BAL) specimens by immunofluorescence (IF) staining techniques. Samples are fixed onto a microscope slide and virus-infected cells are stained with a fluorescein labelled monoclonal antibody directed against the viral antigens. Results can be available within a few hours of sample collection.

The value of this investigation is limited by difficulties with obtaining good quality NPA specimens from adults and the need for invasive techniques to obtain BAL specimens and the subjective nature of IF.

RESPIRATORY VIRUSES

5.2.1.2 Virus isolation

The virus can be grown in cell culture, useful cell lines are PLC and Madin Darby Canine Kidney (MDCK). This method is useful for samples such as respiratory secretions and nose/throat swabs. Infected cells can be identified either by cytopathic effect (CPE) or by haemadsorption. The presence of the virus can then be confirmed by IF.

Virus isolation has for many years been the mainstay of influenza diagnosis, however it relies on viable virus being present in the sample and it may take several days for the virus to grow.

More rapid isolation techniques have been developed and there are now a number of commercially available systems which allow for the detection of virus within 1-3 days⁹³.

5.2.1.3 Point of care test (POCT)

There are a number of commercial assays that are rapid and simple to perform, using respiratory secretions or throat swabs, aimed at clinical teams wishing to provide bedside diagnosis. These assays may also be used in laboratories situated away from virological centres. The POCT assays vary considerably in their sensitivity and specificity. They can be useful adjuncts to diagnosis so long as their limitations are understood by the user. However the routine use of POCT outside of a laboratory setting requires careful attention to quality assurance and training issues.

5.2.1.4 Molecular assays

Detection of the viral genome from the primary sample is now possible using molecular techniques. These assays are sensitive and specific and are increasingly available for routine diagnosis of influenza infection. The majority of these techniques are based on PCR methodology. In generic assays the target is usually internal genes such as the nucleoprotein or the matrix gene. These genes tend to have highly conserved regions within influenza types.

Molecular assays also have the potential to be multiplexed, so that several different target primers, from different viral genomes, can be combined, thereby allowing different pathogens to be identified simultaneously⁹⁴.

Molecular assays have the advantage of being sensitive and specific and can use a number of different specimen types. They are often, however more expensive and require specific training.

5.2.1.5 Serological techniques

Serological methods are still used in many laboratories. They identify the presence of antibody to influenza infection. This can be achieved in a number of ways, such as the detection of a four fold rise in antibody titre, using a group specific antigen in the complement fixation test on paired acute and convalescent sera or by the detection of antibody using enzyme immunoassays. Immunoassays have the advantage of measuring specific classes of immunoglobulins.

Haemagglutination-inhibition assays were once the mainstay of serological investigations. These methods allow detection of antibody to the different subtypes of influenza virus; it is a complex assay and its use is now confined to reference laboratory facilities.

The use of serological investigations is limited by the fact that they detect antibody, which takes several days to develop. They can be used to confirm suspected infection retrospectively, but they do not play a major role in diagnosis during acute infection.

5.3 CLINICAL SYMPTOMS

Influenza virus gains entry to the host via the mucous membranes, the initial replication site being the tracheobronchial ciliated epithelium, and causes a broad range of illness. Classic infection with influenza A follows a short incubation period of 2 days (range 1-4 days). Illness is characterised by the sudden onset of systemic symptoms such as chills, fever, headache, myalgia and anorexia. Respiratory symptoms are common but are often less prominent than

RESPIRATORY VIRUSES

the systemic effects. The main physical findings are pyrexia which can reach 41° C within 12 hours of onset. Fever usually lasts about 3 days, during which time the systemic symptoms are predominant. As the fever declines the respiratory symptoms become more prominent and may include laryngitis and tracheobronchitis; pharyngitis is also common. Adults commonly have systemic illness without respiratory symptoms and children may present with febrile convulsions.

Approximately one third of patients will suffer only mild illness similar to a common cold and it has been estimated that roughly 20% of cases are subclinical. Symptoms are often followed by a long convalescence, with lassitude and malaise lasting up to two weeks^{95,96}.

Considerable variation exists in the severity of the infection and this may be influenced by factors such as age, general health and immune status. Most influenza epidemics and pandemics are associated with an excess mortality, usually in those 65 and over (the exception being the 1918-1919 when individuals in their twenties to thirties were affected). These deaths are largely due to complications such as primary viral pneumonia, secondary bacterial pneumonia (*Staphylococcus aureus* pneumonia is particularly associated with influenza infection) and exacerbation of underlying chronic conditions such as chronic obstructive pulmonary disease or congestive heart disease.

In infants, infection with influenza resembles other respiratory infections and children may present with bronchiolitis, febrile convulsions and occasionally encephalitis. Other complications such as otitis media may follow infection.

Influenza B presents with a similar spectrum of illness to influenza A, although the overall severity is thought to be milder.

5.4 TRANSMISSION

There is considerable debate about the mode of transmission of influenza virus. The possible modes of transmission are by direct contact, indirect contact, droplet or other airborne spread; droplet and direct contact predominate. The part that aerosols play in transmission remains uncertain; it is reasonable to follow airborne precautions in high risk settings^{97,98}.

5.5 EPIDEMIOLOGY

Currently, there is a great deal of confusion with regards to the terminology used to describe influenza infection. For the sake of clarity some common terms are defined below:

Seasonal Influenza: Every year, during the winter period, influenza virus circulates in the community; this subtype of virus, commonly referred to as seasonal influenza, is the latest variation of the current influenza strain differing from the last strain by antigenic drift only.

Pandemic Influenza: This is a global epidemic of a “new” influenza virus. It is a novel viral subtype that has developed through antigenic shift. Influenza pandemics have occurred regularly throughout history, the last of which occurred in 1968.

Avian Influenza: This is an infection of birds. There have been rare cases of human infection with an avian influenza virus in individuals who have had close contact with birds. Recent human infections with an avian virus have raised concerns that a human pandemic may occur as a result of a novel subtype arising from the mixing of a human influenza virus with an avian influenza virus.

5.5.1 INCIDENCE OF INFLUENZA

Epidemics and seasonal influenza tend to occur during a six to eight week period between December and March in the northern hemisphere and May to September in the southern hemisphere. The timing, extent and severity of seasonal influenza can vary. The typical epidemic begins abruptly, peaks at 2-3 weeks and declines by 5-6 weeks⁹⁶.

Approximately 5% of adults and 20% of children develop symptomatic influenza A or B each year⁹⁵.

RESPIRATORY VIRUSES

Influenza activity is associated with an excess mortality. Death rates reflect a combination of two underlying epidemiological parameters: the attack rate of the virus, which is a measure of the pathogen's transmissibility and the case fatality rate which is a measure of the pathogen's virulence. These vary each season depending upon the subtype of the virus circulating and the immunity of the population⁹⁹.

At the present time there are three circulating viruses in the UK, H1N1, H3N2 and influenza B. There is some evidence that H3 subtypes evolve more rapidly than either influenza B or H1 subtypes and cause more severe illness⁹⁵.

5.5.2 PANDEMIC INFLUENZA

The twentieth century saw three pandemics, in 1918, so called "Spanish flu", which was caused by H1N1 virus, in 1957, Asian influenza (H2N2) and in 1968 Hong Kong 'flu (H3N2). These varied in their severity and mortality. The most significant of these pandemics was Spanish flu which resulted in an estimated 40 million deaths. 50% of deaths in this pandemic occurred in the 20-40 year old age group and the case mortality rate was 2.5%¹⁰⁰.

5.6 PREVENTION AND CONTROL

5.6.1 PERIOD OF INFECTIVITY

Individuals infected with influenza are regarded as being infectious for one day before the onset of symptoms and up to 5 days after the onset of symptoms. Children are regarded as being infectious for up to 7 days and can shed the virus for several days before becoming ill.⁹⁵

5.6.2 INFECTION CONTROL

Standard infection control principles and droplet precautions should be rigorously followed. In certain circumstances these control measures may need to be augmented with higher levels of respiratory protection. This may be appropriate in clinical areas where aerosol generation is possible, such as the use of artificial ventilation. Scrupulous attention to handwashing and containment of respiratory secretions produced by coughing and sneezing are the cornerstones of effective infection control.

Detailed guidance on infection control in the case of pandemic influenza has been issued by the Department of Health.¹⁰¹

5.6.3 VACCINATION

The influenza virus is ever changing. Therefore, in order for the vaccine to be effective it must contain the current circulating strain of virus. WHO monitors influenza viruses throughout the world and each year recommends the strains that for the forthcoming winter.

Current vaccines are inactivated, trivalent vaccines, containing two subtypes of influenza A and one of influenza B¹⁰². The viruses are grown in chicken embryos, inactivated chemically and then further treated and purified. Three types of influenza vaccine are currently available:

- 'Split virion, inactivated' or 'disrupted virus' vaccines containing virus components prepared by treating whole viruses with organic solvents or detergents
- 'Surface antigen, inactivated' vaccines containing highly purified haemagglutinin and neuraminidase antigens prepared from disrupted virus particles
- 'Surface antigen, inactivated, virisome' vaccines containing highly purified haemagglutinin and neuraminidase antigens prepared from disrupted virus particles reconstituted into virisomes with phospholipids

All the vaccines are equivalent in efficacy and adverse reactions.

The vaccines give 70-80% protection against infection with influenza virus when the circulating strains are matched to those in the vaccine. Protection against infection derived

RESPIRATORY VIRUSES

from vaccine is estimated to last 12 months protection develops in the individual from 10-14 days after vaccination.

In the UK vaccination is offered to selected groups which are at greater risk of serious morbidity and mortality. These groups include individuals greater than 65 years or with chronic respiratory or cardiac disease. There is considerable interest and research activity looking at the developments of new vaccines for the prevention of influenza. The difficulties of developing influenza vaccines are summarised in Nature Reviews¹⁰³.

5.6.4 ANTIVIRALS

There are limited antiviral agents available against influenza viruses. Three classes of drugs have activity against the virus:

- Inhibitors of M2 (Amantadine and Rimantadine).
- Neuraminidase inhibitors (NI) (Oseltamivir, Zanamivir and Peramivir)
- Ribavirin

5.6.4.1 M2 blockers

These drugs act by interfering with the uncoating of the virus inside the cell by blocking the ion channel formed by the M2 protein.

Amantadine is the only one of this class of drugs which is licensed for use in the UK. It has activity against all influenza A subtypes which have caused human disease. It does not have any activity against influenza B, as the M2 protein is unique to influenza A.

These drugs cause significant side effects, such as nausea, diarrhoea, anorexia, hallucinations and dizziness. Amantadine has been associated with the rapid emergence of drug resistant variants and has a limited role to play in the management of influenza infections.

5.6.4.2 Neuraminidase inhibitor (NI)

These drugs act by inhibiting the neuraminidase enzyme. This enzyme cleaves sialic acid residues on newly formed virions and is essential for release and spread of progeny virions. It is active against influenza A and B.

Oseltamivir (Tamiflu) is taken orally, where it is rapidly absorbed from the gastrointestinal tract and converted to its active metabolite by the liver. This drug is licensed for use in children. It can cause gastro-intestinal side-effects, such as nausea. There is evidence to suggest that certain strains are now resistant to this drug¹⁰⁴.

Zanamivir (Relenza) is taken by inhalation and it licensed for use in children >12 and adults. This drug is well tolerated, although its usefulness is limited by its route of administration.

Peramivir is the latest neuraminidase inhibitor and is not yet licensed for use.

5.6.4.3 Treatment of Influenza by neuraminidase inhibitors

There is good evidence that NI reduce the duration of influenza symptoms by 0.5 -1.5 days in healthy individuals. There is limited evidence for the benefit of treatment in high risk populations and more study is required in this area¹⁰⁵, however it is currently recommended for high risk groups in NICE guidance¹⁰⁶.

5.6.4.4 Use of NI in prophylaxis for Influenza

There is evidence that NIs are useful in preventing the spread of influenza in household contacts, significantly reducing the risk of infection by 70-80%. This reduction may be even higher in residential care facilities where the population has already received vaccination¹⁰⁵.

Comprehensive guidance has been issued on use of antiviral agents in the treatment and prevention of influenza infection in seasonal influenza (NICE)¹⁰⁵ and WHO in the event of human sporadic cases of avian influenza¹⁰⁷.

RESPIRATORY VIRUSES

6 INFLUENZA VIRUS TYPE C

6.1 BASIC VIROLOGY AND TAXONOMY

Influenza C virus was first isolated in 1947. It has been the subject of much less research than influenza A and B viruses due to the relative difficulty in its detection historically and a perception that it is of much less clinical importance.

Influenza C viruses comprise one of the three genera of the family *Orthomyxoviridae*. The nucleoprotein (NP) is type specific. In contrast to influenza A and B viruses there is one surface glycoprotein with both receptor and cleavage activities, often known as the haemagglutinin esterase (HE). Influenza C viral genomes have seven gene segments rather than the eight segments found in influenza A and B viral genomes.

6.2 LABORATORY DIAGNOSIS

Research has increasingly applied PCR, often in a multiplex format to the detection of numerous respiratory viruses from samples from patients with respiratory tract disease, and some of these workers have targeted influenza C virus¹⁰⁸. PCR techniques have higher sensitivity than culture which can be performed in MDCK or Vero¹⁰⁹.

6.3 CLINICAL SYMPTOMS

Influenza C virus causes sporadic upper respiratory tract illness and is perceived as rarely associated with severe lower respiratory tract disease. Administration of influenza C virus to volunteers induced mild coryza with some systemic symptoms. A study of adults with the common cold using RT-PCR detected influenza C virus in 7 of 200 subjects, and this was associated with a rise in specific antibody¹¹⁰. A study in children in hospitalised children in France during October to March also using RT-PCR identified no influenza C virus in 203 nasal aspirates¹⁰⁸. During an epidemic of influenza C virus in Japan up to 2.5% of samples from children with acute respiratory tract symptoms were positive¹⁰⁹. The largest case series published, of 170 culture confirmed cases in children less than 16 years old found that 90% had fever, 74% cough and 62% rhinorrhoea¹¹¹. Of these 92% were aged less than 6 years, 17% (29/170) were hospitalised and 21 had pneumonia or other lower respiratory infection. None died or needed oxygen therapy. On the basis of this experience the authors questioned the perception of influenza C virus as being of low pathogenicity. They did note that the temperature peak was lower and the duration of fever shorter than for influenza A virus infected children.

6.4 EPIDEMIOLOGY

Humans are considered to be the natural host. Antigenic drift is seen but less marked than in influenza A or B viruses. Genetic re-assortment occurs frequently among strains¹⁰⁹. Most people have antibodies to influenza C virus by adulthood. There is no vaccine to influenza C virus. In temperate climates the virus is most prevalent in winter and spring.

6.5 ANTIVIRAL THERAPY

Not susceptible to amantadine (unlike influenza A viruses) or to the neuraminidase inhibitors oseltamivir and zanamivir (unlike influenza A and B viruses).

7 PARAINFLUENZA

7.1 BASIC VIROLOGY

Human parainfluenza Virus (HPIV) is in the *Paramyxoviridae* family, a pleiomorphic enveloped virus, with a single, negative strand RNA genome. Medium-sized: 150-250nm diameter. HPIV is genetically and antigenically divided into types 1 through 4. The majority of structural and biological attributes are similar but each is adapted to different times of year and infections of different age groups as well as having different possible clinical presentations¹¹².

RESPIRATORY VIRUSES

7.2 LABORATORY DIAGNOSIS

Laboratory diagnosis may be by virus isolation, antigen detection, or molecular methods. Electron microscopy can confirm HPIV, but it is impossible to distinguish electron micrographs of HPIV from other paramyxoviruses (eg mumps).

Most commonly, HPIV is detected by direct examination of NPA using commercially available type-specific fluorescein conjugated monoclonal antibodies. Increasingly, molecular detection by reverse transcriptase polymerase chain reaction (RT-PCR) is used as it can be applied to a wider range of specimen types.

Appropriate specimens include throat or nasopharyngeal swabs, nasal washes and nasal aspirations. For culture, specimens should be collected and placed in viral transport media with antibiotics and antifungals, buffered to pH 7.5-8.0 and kept at 4 °C until inoculation. If this is to be delayed for more than 24 h, the specimen should be frozen. Specimens may be centrifuged at 1000 g prior to inoculation to remove any debris.

HPIV are isolated more readily in epithelial cell lines than fibroblast cell lines. Cell lines that support growth include LLC-MK2, Vero, CV-1, Hep-2, BHK and HeLa.

CPE is rarely observed in primary isolation of HPIV, so is not reliable to detect positive cultures. Haemadsorption of guinea pig RBCs can usually allow detection of HPIV-1 and HPIV-3 within days, but HPIV-2 and HPIV-4 take longer¹¹³.

Shell vials allow rapid identification, but again, sensitivity is not high. Direct immunofluorescence is very specific but not very sensitive, although use of monoclonal antibodies can increase sensitivity.

There is insufficient viral RNA in clinical specimens to detect without amplification, either by culture or nucleic acid amplification. Nucleic acid tests include commercially available multiplex and real time RT-PCR assays.

For serological confirmation, acute phase sera should be collected as early into the illness as possible. The second specimen should be drawn at 3-5 weeks after infection. Specimens should be frozen immediately (-20 °C or -70 °C) and acute and convalescent sera tested at the same time.

7.3 CLINICAL SYMPTOMS

Symptoms can include; fever, croup (especially children aged 1-2 years), for which HPIVs are the most common aetiological cause, bronchiolitis (especially from HPIV-3), pneumonia (including presentation with secondary bacterial infections), tracheobronchitis, otitis media (with or without additional bacterial infection). In 5-20% of lower respiratory infections, more than one virus can be detected and there may be more severe disease. HPIV-1 is thought to be responsible for at least 50% of autumn croup cases in the USA.

7.4 TRANSMISSION

Close contact and surface contamination (demonstrated survival 10 h on non-porous material; 4 h on porous).

7.5 EPIDEMIOLOGY

HPIVs are generally considered community acquired respiratory pathogens. They have a worldwide distribution. HPIVs are estimated to cause up to one third of all respiratory tract infections and up to half of all respiratory infections in pre-school children, boys being affected more than girls. HPIV-1 to 3 are also major causes of respiratory illness in patients who are immunocompromised, elderly and chronically ill. Less is known about HPIV-4. Young infants seem particularly susceptible to HPIV-1 and HPIV-3.

RESPIRATORY VIRUSES

HPIV-1 causes large seasonal outbreaks every other year, and HPIV-2 outbreaks usually follow those of HPIV-1. In contrast, HPIV-3 generally causes annual outbreaks in the spring or summer.

Humoral immunity is important, especially to HN and F glycoproteins. Repeated infections are required for immunity.

7.6 PREVENTION AND CONTROL

Treatment is generally supportive, requiring maintenance of airway and hydration. Steroids can be beneficial in treatment of croup. Although there are some reports of interferons being effective against parainfluenza most reports of antiviral use in severe parainfluenza infection, particularly in the transplant setting, describe the use of ribavirin.

No vaccinations have yet proved successful.

8 RHINOVIRUS

8.1 BASIC VIROLOGY

Rhinoviruses are members of the *Picornaviridae*, a large group of animal viruses. Picornaviruses are icosahedral, approximately 30nm in diameter, non-enveloped, positive-sense single-stranded RNA viruses. The family is divided into five genera based on genetic sequence information. These are the enteroviruses, hepatoviruses, rhinoviruses, cardioviruses and aphthoviruses. A number of clinical, epidemiological and biophysical properties distinguish rhinoviruses, which inhabit the upper respiratory tract, from enteroviruses, which are found throughout the gastrointestinal tract. For instance, rhinoviruses are unstable below pH 6, whereas enteroviruses are stable at pH 3 -10¹¹⁴. Variation of capsid proteins containing viral genome produce >100 distinct human rhinovirus (HRV) serotypes¹¹⁵. A number of studies have confirmed HRV as being the most common cause of viral respiratory infections (VRI).

8.2 LABORATORY DIAGNOSIS

The diagnosis of rhinovirus has traditionally been by cell culture followed by acid lability testing. Serology is complicated by more than 100 serotypes¹¹⁶. More recently these techniques have been superseded by the use of molecular techniques most commonly PCR. Respiratory samples used include nose and throat swabs, nasopharyngeal aspirates, nasal washouts and sinus brushings¹¹⁷. However the most significant diagnostic tool is the multiplex PCR. One method by Grohndal et al (1999)¹¹⁸ described the rapid isolation of nine microorganisms causing acute respiratory tract infection in a single tube multiplex RT-PCR.

There have been increasing numbers of reports of rhinovirus detected by PCR in respiratory samples from transplant patients .

8.3 CLINICAL SYMPTOMS

The host response to the cold virus is believed to be the major cause of cold symptoms^{119,120}. As the cold progresses, the virus moves more anteriorly in the nares. The prominent symptoms of rhinorrhoea and nasal obstruction result from increased vascular permeability, with leakage of serum into the nasal mucosa and nasal secretions. Cold symptoms are also caused by neurogenic reflexes triggered by the infection. The inflammatory mediators interleukin (IL)-1 α , IL-6, IL-8 have been found in the nasal secretions of symptomatic subjects and are responsible for symptoms¹²⁰. Symptoms occur with 16h of inoculation and peak from 24 – 48 h post inoculation. The virus can be recovered after 24h and shedding peaks on days 2 - 3¹²¹. Viral shedding persists after the resolution of symptoms, and the virus may be cultured from 10 – 20% of subjects 2 -3 weeks after infection. Sore throat is usually the first sign of infection and then in addition to rhinorrhea and nasal obstruction, facial or sinus pressure, headache and cough are also common. Non productive cough can persist for a week and it may be more protracted in smokers. In adults fever is uncommon.

RESPIRATORY VIRUSES

8.4 TRANSMISSION

Transmission of HRV is most efficient by direct contact, although infections have been documented by both large and small particle aerosols regardless of the route; initiation of infection occurs when contact is made between the virus and nasal mucosa. Typically HRV transmission occurs in the home with a child attending day care or school being the most frequent introducer of the infection.^{115,122} Infection is spread from hand to hand contact with contaminated nasal secretions, usually from child to mother. Self inoculation from eye rubbing or nose picking also spreads infection^{120,123}.

8.5 EPIDEMIOLOGY

In studies carried out by Arruda et al rhinovirus was found in 80% of patients who had upper respiratory infections and colds between September and October¹²⁴. Other studies have confirmed high prevalence of HRV in VRI and seasonal peaks in autumn and early spring (March and April)¹²⁵.

Complications of HRV are not uncommon and they include otitis media, sinusitis, acute exacerbations of asthma and COPD and exacerbations of cystic fibrosis. The airway hyperreactivity mechanisms triggered by rhinovirus infection are not completely understood, but contributing factors may include increases in vagally mediated reflex bronchoconstriction, the release of immunologic mediators, increased airway responsiveness to tachykinins, recruitment and activation of inflammatory cells and the induction of IgE class switching¹¹⁵. HRVs have an important clinical role in precipitating asthma attacks. Johnston and colleagues¹²⁶ reported that amongst children 9 to 11 years of age, common cold viruses were present in 80 to 85% of asthma exacerbations. HRVs were the most common pathogen in this group being detected in 66% of the viral isolates. Other studies show that HRV infection in children is associated with high rates of wheezing and asthma related emergency department admissions¹¹⁵. In adults studies have shown that HRV infection was associated with 40 to 43% of exacerbations experienced by patients with chronic bronchitis¹²³. Outbreaks of HRV among elderly persons in the community and in nursing homes are important causes of respiratory illness. Nicholson et al¹²⁷ reported that chronic illness increased the likelihood of HRV associated lower respiratory tract illness by 40% and that smoking increased the risk by 47%. Those with underlying pulmonary disease had more prolonged and more and more severe illness than those without lung disease.

8.6 PREVENTION AND CONTROL

There are currently no vaccines for rhinoviruses. There are several treatment strategies with varying efficacy¹²⁸.

9 RESPIRATORY SYNCYTIAL VIRUS

9.1 BASIC VIROLOGY

Respiratory Syncytial Virus (RSV) was originally isolated in 1956 from a colony of chimpanzees with coryza and was designated chimpanzee coryzal agent¹²⁹. Subsequently RSV was found to be an enveloped pleomorphic virus, diameter of 150 – 300 nm, with a negative sense, single stranded genome encoding 10 proteins, 2 of which are non-structural. The virus has been classified within the *Paramyxoviridae* family, belonging to the *Pneumovirus* genus. A and B subgroups have been identified¹³⁰. Both strains circulate concurrently with the A strains usually predominating.

9.2 LABORATORY DIAGNOSIS

The most common method for detecting RSV in clinical specimens is immunofluorescence using commercial monoclonal antibodies. Suitable specimens include nasopharyngeal aspirates, nasal washes and bronchoalveolar lavage. The virus can be isolated in cell cultures, commonly heteroploid cell lines, such as Hep-2 or HeLa cells. A cytoplasmic effect, of a syncytial (giant cell) nature, can be seen usually after 3 to 5 days. Recently reverse transcriptase PCR methods have been introduced into some laboratories. Serological

RESPIRATORY VIRUSES

diagnosis is less commonly used but may be useful in retrospectively identifying outbreaks in institutions for the elderly, using the complement fixation test.

9.3 TRANSMISSION

RSV is transmitted both by direct contact with secretions or fomites or by spread of large droplets. Volunteer inoculation studies have shown that virus transmission was easily achieved via the nose and eyes but not into the mouth. Small particle aerosol transmission is uncommon. Virus has been recovered from environmental surfaces in patients' rooms for up to 6 hours.

9.4 EPIDEMIOLOGY

In temperate zones RSV usually circulates during the winter causing annual epidemics, typically between November and March. RSV predominantly causes significant illness in young infants and elderly people. It affects approximately 90% of infants and young children by the age of 2 years, with a peak incidence in infants aged 6 weeks to 6 months. In the elderly, at least 10% of hospitalised winter admissions are caused by RSV with a case-fatality rate of about 10%, similar to that of Influenza in this population¹³¹, RSV is also an important cause of community-acquired lower respiratory infection among hospitalised adults¹³² although this is often overlooked by clinicians.

9.5 CLINICAL SYMPTOMS

In the general population RSV commonly causes infection of the upper respiratory tract resulting in rhinitis, cough and sometimes fever. However in young children it is a significant pathogen causing 50 to 90% of hospitalisations for bronchiolitis, 5 to 40% for pneumonia and 10 to 30% for tracheobronchitis¹³³. Acute otitis media occurs in up to a third of children with RSV illness and both RSV and bacterial pathogens have been isolated from middle ear fluid of children with RSV. Recurrent wheezing can be common after RSV bronchiolitis and has been reported in 10 to 50% of former patients¹³⁴ but is rarely of great severity. Infants with underlying cardiac or respiratory disease are prone to severe disease and may require admission to intensive care wards. However nowadays the overall mortality rate is low occurring in less than 2% of hospitalised infants. Re-infection in both children and adults is common as mucosal immunity is incomplete and of short duration¹³⁵. Re-infection in adults, especially in the elderly, is often moderately severe. Children and adults who are immunocompromised are vulnerable to RSV infection. Severe problems may be encountered in bone marrow transplant patients, particularly if infection occurs early post-transplant, prior to bone marrow engraftment and mortality rates may be high¹³⁶. Often the majority of these infections are hospital acquired and outbreaks can occur if infections are not rapidly recognised and effective infection control measures are not implemented.

9.6 PREVENTION AND CONTROL

A vaccine against RSV is highly desirable given the significant morbidity and widespread nature of this virus. In the 1960s enhanced disease was seen in naïve infants vaccinated with formalin-inactivated virus¹³⁷. Subsequently it was found that bronchiolitis caused by RSV is an immune mediated process and that the vaccine had elicited an exaggerated destructive immune response. Not surprisingly this resulted in extreme caution being exercised in subsequent RSV vaccine trials. A number of experimental vaccines have been trialled over the years but a successful, safe vaccine is still awaited. Ideally any new vaccine would need to elicit more durable immunity than that given by natural infection as re-infection is so common. A vaccine would need to be very immunogenic as the vaccine would need to be given at or around the time of birth. Monthly administration of commercial preparations of RSV hyperimmune globulin or recombinant monoclonal antibody against the fusion protein of RSV (palivizumab), in premature infants or infants with chronic lung disease, significantly reduced the risk of subsequent hospitalisations for RSV infection¹³⁷. Only palivizumab (Synagis ®) is licensed in the United Kingdom for prophylaxis of RSV infection and is given intramuscularly on a monthly basis for around 5 months during the RSV season to selected infants and children eg children under 2 years of age with chronic lung disease. Currently the only therapy for RSV infection is aerosolised ribavirin, a synthetic guanosine analogue which is approved only for hospitalised infants. However studies on the efficacy of ribavirin have shown conflicting results. A meta-analysis of these studies, in infants with RSV lower

RESPIRATORY VIRUSES

respiratory tract infection, showed that there was no evidence of a significant benefit, and that studies lacked the power to detect reductions in mortality. Given the unproven efficacy and expense of ribavirin, in practice it is only used in infants with severe disease. A large amount of evidence has shown that immunopathological mechanisms are, in part, responsible for the symptoms of RSV bronchiolitis. Consequently in theory, corticosteroids may be an effective treatment. Not surprisingly this suggestion has been controversial. However a meta-analysis of systemic corticosteroids in infant bronchiolitis suggested a statistically significant benefit in clinical symptoms. Although this approach is seldom used, it appears to be effective in children with severe combined immunodeficiency with RSV infection of the lower respiratory tract, post bone marrow transplantation, when combined with the use of ribavirin.

10 OTHER RESPIRATORY VIRUSES

10.1 HANTAVIRUS INFECTION

The Sin Nombre virus is a hantavirus¹³⁸ which was discovered through investigation of an outbreak of respiratory disease in that part of the USA where the four states of New Mexico, Arizona, Utah, and Colorado meet. The disease came to be known as hantavirus pulmonary syndrome (HPS). The natural host for the virus is the deer mouse *Peromyscus maniculatus*; humans are infected through contact with infected rodent excreta. HPS is a rapidly progressing pulmonary disease with a fatality rate of up to 50%. The initial presentation is with the sudden onset of fever and generalized myalgia, often accompanied by abdominal pain, nausea and vomiting. After 4-5 days respiratory symptoms appear with cough, tachypnoea and tachycardia. Rhinorrhoea and sore throat are seldom seen. Mild hypotension is common. Vascular leakage occurs and the lung disease may progress from mild desaturation with interstitial pulmonary oedema to marked pulmonary oedema and respiratory failure. Thrombocytopenia is a feature, together with atypical lymphocytes and raised liver aminotransferases^{139,140}. Cases of HPS have also been caused by other North American hantaviruses – New York virus, Black Creek Canal virus, and Bayou virus – but Sin Nombre virus accounts for over 95% of cases in the USA.

HPS is more prevalent in South America, where the principal hantavirus involved is the Andes virus, which has caused outbreaks in Argentina, Chile and Uruguay^{141,142}. Laguna Negra virus (carried by the vesper mouse *Calomys laucha*) has caused outbreaks in Paraguay. In addition to infection with Andes virus from carrier rodents (notably *Oligoryzomys longicaudatus*) person to person transmission has been demonstrated¹⁴³.

In Europe pulmonary involvement in Puumala virus infection is not infrequent but is seldom severe¹⁴⁴.

10.1.1 DIAGNOSIS OF HANTAVIRUS INFECTION

Serological diagnosis can be made by testing for IgM antibody by immunoassays such as ELISA and immunoblot and by demonstrating seroconversion or four-fold rise in IgG antibodies. Anti-G1 antibodies are hantavirus type-specific. Hantavirus IgM is generally present even as early as the prodromal phase of the illness^{145,146}. Immunohistochemistry can be used on fixed lung tissue to make a diagnosis of hantavirus infection. PCR can be used on tissue, whole blood and serum, followed by sequencing to identify the virus strain¹⁴².

10.2 NIPAH VIRUS

Nipah virus is a highly pathogenic paramyxovirus grouped together with the Australian Hendra virus as the henipaviruses. Nipah virus causes infection in humans and in pigs in SE Asia. The henipaviruses are agents of zoonotic infection with their natural reservoir *Pteropus* fruit bats (flying foxes). Nipah virus emerged in 1998 in Malaysia as a cluster of cases of encephalitis in pig farmers and spread to Singapore. The original outbreak of neurological illness was preceded by respiratory illness and encephalitis in pigs in the same area; humans were infected by close contact with infected pigs. In more recent outbreaks in Bangladesh direct transmission from bats as well as human to human transmission has been noted¹⁴⁷. Although the principal feature of human Nipah virus infection is encephalitis some patients (up to 25%) do have respiratory symptoms and the chest X-ray is abnormal in up to 10% of cases¹⁴⁸.

RESPIRATORY VIRUSES

10.2.1 DIAGNOSIS OF NIPAH VIRUS INFECTION

Diagnostic tests should only be carried out at a Containment level 4 facility.

Nipah virus can be found early in the illness in respiratory secretions and in urine¹⁴⁹. It grows readily in a range of cells including Vero cells, in which a syncytial CPE is seen at 5-7 days post-inoculation^{148,149}. Nose swab was more sensitive than throat swab in the 1998 outbreak¹⁴⁹.

Serological diagnosis employs immunoassays for IgG and IgM antibodies to Nipah virus^{150,151}. As specificity of EIA testing is less than 99% ELISA reactive tests need confirmation by a neutralization test.

Diagnostic Nipah virus PCR tests can be used on CSF or other specimens including respiratory samples. A range of PCR methods have been used in various centres, employing primers to M, N, or P genes¹⁵⁰. Immunohistochemistry can be used to provide a retrospective diagnosis on fixed tissues including brain and lung¹⁵⁰.

11 RELEVANT NATIONAL STANDARD METHODS

For additional details on specific areas of diagnosis refer to the relevant NSMs available through the Evaluations and Standards Laboratory web page (www.hpa-standardmethods.org.uk).

Other documents of relevance to this NSM are:

[VSOP 18 - Complement fixation tests](#)

[VSOP 22 - Immunofluorescence and isolation of viruses from respiratory samples](#)

[VSOP 45 - Haemadsorption of viruses](#)

RESPIRATORY VIRUSES

Issue no: 1 Issue date: 08.08.08 Issued by: Standards Unit, Evaluations and Standards Laboratory Page no: 28 of 38
QSOP 60i1

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RESPIRATORY VIRUSES

Issue no: 1 Issue date: 08.08.08 Issued by: Standards Unit, Evaluations and Standards Laboratory Page no: 29 of 38
QSOP 60i1

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Issue no: 1 Issue date: 08.08.08 Issued by: Standards Unit, Evaluations and Standards Laboratory Page no: 30 of 38
QSOP 60i1

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RESPIRATORY VIRUSES

Issue no: 1 Issue date: 08.08.08 Issued by: Standards Unit, Evaluations and Standards Laboratory Page no: 31 of 38
 QSOP 60i1

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RESPIRATORY VIRUSES

Issue no: 1 Issue date: 08.08.08 Issued by: Standards Unit, Evaluations and Standards Laboratory Page no: 32 of 38
 QSOP 60i1

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Issue no: 1 Issue date: 08.08.08 Issued by: Standards Unit, Evaluations and Standards Laboratory Page no: 33 of 38
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