MINI-SYMPOSIUM: MICROBIOLOGICAL DIAGNOSTIC PROCEDURES IN RESPIRATORY INFECTION

Respiratory virus infections

Alison M. Kesson*

*Department of Infectious Diseases and Microbiology, The Children's Hospital at Westmead,
Discipline of Paediatrics and Child Health, University of Sydney, LMB 4001, Westmead NSW 2145, Australia*

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Summary The respiratory tract is a frequent site of infection with a wide range of viruses. Each family of viruses can cause differing clinical syndromes depending on the age of the patient and the immune response. As a corollary, different clinical syndromes can be caused by different families of viruses.
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INTRODUCTION

Respiratory virus infections represent a major public health problem because of their worldwide occurrence, ease of spread in the community and considerable morbidity and mortality. Mortality from respiratory virus infections in healthy individuals in developed countries is rare; however, in less developed countries childhood mortality can be quite high with an estimated 5 million children globally under 5 years dying annually from respiratory virus infections. Their worldwide occurrence results in people of all ages being susceptible to respiratory virus infections. However, on average, children are infected two to three times more frequently than adults, with acute respiratory virus infections being the most common infections experienced by healthy children and often resulting in loss of school time and a significant socioeconomic cost in medical visits, medications and parents' loss of work time.

Most viruses are transmitted by direct contact or droplets, although some are transmitted by aerosols. Viruses that primarily infect the respiratory tract include influenza, adenoviruses, parainfluenza viruses, respiratory syncytial virus (RSV), coronaviruses, human metapneumovirus, rhinoviruses and enteroviruses. More recently, a new virus, bocavirus, has been identified. Influenza, parain-fluenza, human metapneumovirus (hMPV) and RSV occur in epidemics while adenovirus, coronavirus and rhinoviruses occur endemically. Other viruses which can cause respiratory diseases, more commonly in immunosuppressed people, include measles, varicella zoster virus (VZV), herpes simplex virus (HSV) and cytomegalovirus (CMV).

Aetiological diagnosis of viral respiratory tract illness requires laboratory confirmation as the symptoms and signs of the clinical illness lack specificity to permit aetiological recognition on clinical grounds alone. Development of safe chemotherapeutic agents and highly immunogenetic and protective vaccines for each of the individual respiratory viruses is a continuing priority: except for influenza virus, this goal remains elusive.

Seasonal occurrence

Respiratory virus infections often have a seasonal distribution, especially in temperate climates, and while the peak incidence varies year to year there is often a predominant seasonal occurrence. RSV and influenza both have a peak incidence in winter and these peaks usually do not coincide but overlap. Parainfluenza virus (PIV) 3 usually peaks in winter with PIV 1 and PIV 2 peaking in autumn and early winter. The Picornaviridae cause infections all year round with enteroviruses more common in summer and autumn while rhinoviruses are more common in winter and spring.

* Tel.: +61 2 98453823; Fax: +61 2 98453291.
   E-mail address: alisonk2@chw.edu.au.

KEY POINTS

- Respiratory virus infections are a major source of morbidity and mortality globally.
- New respiratory viruses with epidemic and pandemic potential continue to be identified.
- Diagnosis of respiratory virus infections continues to improve with new technological methods.

Adenoviruses tend to cause infections year round as do the Herpesviridae, except for varicella which is more prevalent in the late winter and early spring.

VIRUS CULTURE METHODS

Conventional virus culture

Viral culture is the original method used for diagnosing respiratory virus infections and culture for viruses using primary and immortalized cells was expanded for wider diagnostic use in the 1950s. The main advantage of traditional cell culture methods is the ability to isolate and identify a wide range of viruses.[1] This has enabled the identification of many viruses, including those commonly causing respiratory infections – influenza, RSV, PIV 1–4, adenoviruses, measles, enteroviruses, rhinoviruses, VZV, CMV and HSV. More recently recognized viruses such as severe acute respiratory syndrome coronavirus (SARS-CoV) and hMPV have also been grown in cell culture.

For virus isolation to be successful careful attention needs to be paid to a number of issues. These include the selection of appropriate patients, collection of adequate specimens, transport of the specimen to the virology laboratory under optimal conditions and processing methods of the clinical samples.

Respiratory samples for virus isolation include nasopharyngeal aspirates, nasopharyngeal and/or oropharyngeal swabs, nasopharyngeal washes, bronchioalveolar lavages, sputum and lung biopsies. Other than open lung biopsies all other specimens are contaminated with commensal respiratory flora. Dacron or polyester swabs collected from the respiratory tract should be placed into viral transport medium containing antibiotics. The specimens should be kept cool at 2–8 °C or on wet ice until inoculation of the cell cultures to maintain virus infectivity, especially for labile viruses such as RSV.[2]

Detection of viruses in cell culture requires considerable expertise and is performed by microscopic examination looking for degenerative morphological changes in the cell monolayer called cytopathic effect (CPE). Not all viruses grow in all cell types so clinical specimens are usually inoculated into several types of cells to provide an environment for the isolation of a suitable range of viruses. The use of a broad range of cell types potentially allows for the isolation of agents which are not expected rather than limiting detection to only a few virus types. This approach will also allow the detection of more than one virus in any given specimen which can occur in up to 5–10% of immunocompetent individuals and more frequently in immunocompromised patients especially with viruses which have latency such as CMV and HSV. Recently, commercially produced co-cultivated cryopreserved cells have become available for the rapid identification of viruses. R-Mix rapid cell culture (Diagnostic Hybrids) is a patented cell monolayer containing a mixture of A549 and mink lung (Mv1Lu) cells.[3] After 24–72 h of culture, using pooled or single fluorescein isothiocyanate (FITC)-labelled monoclonal antibodies directed against influenza A and B, RSV, parainfluenza 1–3 and adenoviruses, rapid identification of a respiratory virus infection can be established.

Growth of a virus in cell culture indicates the presence of viable, replication competent virus, a finding which cannot be determined using antigen or nucleic acid detection. In addition, the propagation of an isolate provides virus for antiviral susceptibility testing, serotyping and epidemiological studies.

Rapid viral cultures

More recently newer viral culture formats have been developed which allow for more rapid detection of viruses, which is very useful for detection of those viruses which grow slowly in conventional cell culture. Decreasing the time until CPE is detectable has been achieved for many viruses by centrifugation of the culture after the sample has been added. As viruses are unable to be centrifuged with conventional centrifuges, the enhanced detection may result from better contact between cells in the specimen and the cell culture thus allowing for earlier and more extensive infection of the cell culture sheet. Furthermore, the detection of viral protein production (antigen detection) before there is evidence of CPE in conventional culture has also decreased the time to identify a virus and thus enhanced clinical utility of viral culture testing. The detection of viral protein production is usually performed using a fluorescent-labelled (e.g. FITC) monoclonal antibody directed against a viral protein. Processing and reading these cultures is labour intensive; however, most common viruses can be detected in 24–48 h. Alternatively enzyme-linked immunosorbent assay (ELISA) detection of viral proteins in cell culture supernatants has also been performed.

Viral culture with transgenic cells

More recently, transgenic cell lines with virus-induced reportable elements have been developed for use in viral culture. This uses a gene promoter which is 'quiet' in uninfected cells but is sufficiently upregulated by viral transactivator proteins and with sufficient specificity that it does not allow heterologous viral transactivating proteins

to activate the promoter.[4] This technology has been exploited to allow detection of HSV-1 or -2 using the baby hampster kidney (BHK) cell line stably transformed with the promoter of UL39 from HSV linked to the *Escherichia coli* lacZ gene. HSV can be detected within 24 h of infection using chromogenic substrate which turns infected cells from colourless to blue. HSV infected cells are easily identified and this technique could be expanded to determine viral titre in specimens if required. This system is marketed by Diagnostic Hybrids under the name ELVIS (enzyme-linked virus-inducible system).[5]

NON-CULTURE METHODS OF VIRUS DETECTION

Immunofluorescence

The ability to detect viruses was significantly enhanced by the development of monoclonal antibodies (MAb) directed against specific viral proteins for the viruses HSV-1, HSV-2, VZV, CMV, influenza A, influenza B, parainfluenza 1–3, and adenovirus, and linked to the fluorescent molecule FITC. These staining procedures are rapid: samples are batch run and results are usually available within 1–3 h depending on the number of specimens tested. Rapid immunofluorescent (IF) staining methods for the direct detection of viral antigens in respiratory specimens have excellent specificity and very good sensitivity. Most respiratory viruses produce CPE in conventional cell cultures but often this is slow or very minimal in extent. The rapid detection of the respiratory viruses influenza A, influenza B, parainfluenza 1–3 and adenovirus by IF is available in many laboratories and is extremely useful for the detection of these viruses, especially in paediatric patients who shed relatively large amounts of virus and for a longer period than do adults. The performance characteristics of the test depend on the type of specimen, age of the patient, duration of illness, MAb reagent and level of expertise of the laboratory staff. Generally, IF methods for respiratory viruses are a little less sensitive than culture with the exception of RSV. However, the greater sensitivity of RSV IF compared with RSV culture is due to the lability of RSV which is rapidly inactivated in samples that are not kept refrigerated and transported to the laboratory quickly for inoculation into cell cultures within a short period after collection of the specimen. The reported sensitivities of IF detection of respiratory viruses varies but are broadly 90% for RSV, 80% for influenza A and B, 70% for parainfluenza 1–3 and 50% for adenoviruses.[6] MAbs directed against hMPV have been developed and are currently under investigation to establish their clinical utility.

Non-immunofluorescence methods

Membrane-based (cassette-based) enzyme immunoassays (EIAs), optical immunoassays and immunochromographic or lateral-flow systems have been introduced for less technically demanding detection of respiratory viruses.[7] Some can now differentiate viruses, e.g. influenza A from influenza B, RSV from influenza A, and include a positive control to monitor performance of both the assay and the user. A significant aim of the manufacturers of these tests is to allow for testing to occur in the consulting physician's office. These techniques have the advantage of decreasing turn-around time for test results and have considerably simplified the technical component, but these tests are not as sensitive or specific as viral culture or direct IF (DIF) for detection of respiratory viruses. The positive predictive value of these assays is usually higher when there is a greater prevalence of disease in the respiratory virus season and the virus in question has been shown to be circulating in the community by viral culture or IF. During periods of relatively high prevalence positive results can usually be accepted as correct; however, negative results should be confirmed by a second method, e.g. DIF or culture.[8]

Nucleic acid detection

Viruses can be detected in clinical samples using highly specific nucleic acid probes that are complementary in sequence to viral RNAs or viral DNAs, or by using a nucleic acid amplification technique such as polymerase chain reaction (PCR), branch chain DNA detection (bDNA) or nucleic acid sequence based amplification (NASBA). These techniques are becoming increasingly available in diagnostic laboratories but testing for multiple viruses using molecular methods is considerably more expensive than with conventional techniques and the methodology needs to be developed in house. Evidence would suggest that testing using molecular methods is a little more sensitive than conventional approaches but the information available to date is variable.[9]

THE VIRUSES

Influenza viruses

Influenza A virus was isolated in 1933, influenza B in 1940 and influenza C virus in 1951. Influenza A and B viruses belonged to the family Orthomyxoviridae and are in the genus *Influenzavirus*. Influenza C virus belongs to a separate genus. Influenza virions are enveloped particles (i.e. surrounded by a lipid bilayer membrane of cellular origin) containing a single-stranded negative-sense segmented RNA genome that is surrounded by a helical capsid, with influenza A and B containing eight segments of RNA and influenza C containing seven segments. Only influenza A and B are clinically important. Influenza C infection occurs uncommonly and is usually associated with the mild upper respiratory tract illness; rarely it can cause bronchitis or pneumonia.

The enveloped virion has peplomers or spikes consisting of two glycoproteins – haemagglutinin (H) which is involved in the attachment of the virus to cells and the initiation of infection, and neuraminidase (N) which facilitates release of newly formed virions from the cells. The two glycoproteins, H and N, exhibit substantial antigenic variation among influenza A viruses with 16 H subtypes and 9 N subtypes recognized. Influenza B has only one type of H and N glycoproteins. The occurrence of annual influenza epidemics throughout the world is due to the high rate of antigenic variation from a stepwise mutation of the H and/or N genes and reflected in variations of the antigenic characteristics of these proteins (and thus escape from immune memory) by the H and N glycoproteins of influenza A and B viruses. This stepwise mutation of H and N results in antigenic drift and the recurrent annual influenza epidemics seen each winter. The virus can infect and produce disease among populations who would otherwise possess immunity from previous infection because their antibodies fail to recognize the new antigenic variation(s). This is the reason for the necessity for annual influenza vaccination with differing serotypes of influenza A and B viruses.

Additionally, the segmented nature of the influenza genome allows for antigenic shift – the re-assortment of genome segments from two different influenza A viruses with major changes in the H or N proteins or both. It is this major variation in genetic make-up which gives the influenza A virus the potential for the development of global pandemics.

H5N1 influenza A

During the past few years, new influenza viruses isolated in Hong Kong, H5N1, appear to have spread from poultry to a small number of persons, who have shown a high mortality rate. This has caused a high degree of global concern as spread of avian influenza A strains to humans is usually very uncommon but several hundred human cases of H5N1 infection have been identified in countries across the world.[10] The major limitation to this virus causing a pandemic with a huge impact on health and the economy is the failure of the H5N1 influenza A to establish sustained human-to-human transmission. Were this to occur a pandemic would be inevitable. Commonly available routine diagnostic tests in laboratories can differentiate influenza A from influenza B; however, they are unable to identify the subtype. Identification of the strain of influenza can be performed by a variety of methods at reference laboratories if required.[11]

Laboratory diagnosis of influenza virus infection by isolation of the virus in cell culture is the 'gold' standard technique. This technique however is slow and does not allow for diagnosis within the time frame for specific treatment. However, virus isolation remains critically important for epidemiological studies, the recognition of specific types of influenza circulating in the community and identification of new types and thus pandemics. The information on influenza types is used to design vaccines for the upcoming influenza season.

Influenza culture can be performed on nasopharyngeal aspirates, nasopharyngeal swabs, throat swabs and sputum, or lung biopsy tissue or bronchoalveolar lavages. Specimens should be kept at 4 °C until processed. More recently, rapid cultures involving growth of influenza for up to 3 days and then labelling cells with antibodies directed against influenza A using an immunoperoxidase or fluorescence technique have been developed. These techniques are rapid and relatively inexpensive but do require a high degree of technical competency. They can accurately differentiate influenza A or B from other viruses but are not suitable for identifying the H type of influenza A. Thus these tests would not be diagnostic in the early stages of a pandemic when the new H type of influenza A would be co-circulating with the old H type. Direct detection of viral antigen in respiratory specimens can also be performed and is the technique of choice for a rapid sensitive assay. Reverse transcription-polymerase chain reaction (RT-PCR) is now available in many laboratories and can differentiate influenza A and B and other viruses. It can also be used to identify differing H or N types of influenza A.

Detection of an immunological response, with a four-fold or greater rise in serum antibody titres using haemagglutination inhibition or complement fixation techniques when acute and convalescent samples are compared, also provides evidence of acute infection with the influenza A or B virus. Serological tests are rarely diagnostic in individual cases within a timeframe that allows judicious use of anti-influenza drugs but they are very useful for tracking epidemics in the community. ELISA tests for IgM on a single sample are also available. IgM and IgA specific antibodies against influenza peak about 14 days after infection and IgG specific antibody peaks at about 4–7 weeks. Serological diagnosis and typing of influenza virus can be complicated by the fact that the anamnestic response to infection is highest for the strain causing the primary infection, even when there is subsequent infection by other strains. This has been termed the 'doctrine of original antigenic sin' and can lead to a lack of strain specificity with serological tests.

Parainfluenza viruses

Parainfluenza virus types 1, 2 and 3 occur worldwide and among persons from all age groups. Parainfluenza viruses 4A and 4B are much less frequent.

Parainfluenza 1 occurs in epidemics usually during autumn in alternate years, parainfluenza 2 occurs sporadically and parainfluenza 3 tends to cause annual winter epidemics in temperate climates. Parainfluenza 1–3 are the main causes of croup in infants and young children under 5 years of age. Parainfluenza 3 can also cause viral

pneumonia and bronchiolitis in infants and small children. Parainfluenza 4 occurs very infrequently and is usually associated with mild symptoms of upper respiratory tract illness (rhinorrhea, pharyngitis and cough). Primary infection with parainfluenza provides some measure of immunity but this immunity is not complete or long lasting and re-infections occur commonly but they are rarely as severe as the illnesses seen with primary infection.

Laboratory diagnosis of parainfluenza infection is based on viral isolation in tissue culture and this remains the 'gold' standard. Respiratory secretion specimens must be kept at 4 °C until processed. Parainfluenza grows relatively slowly, taking 3–5 days, and as these viruses do not cause a direct CPE, they are detected using haemadsorption (adsorption of guinea pig red blood cells to infected cells). Rapid diagnostic tests for detection of viral antigen in respiratory specimens using DIF are specific for each of the parainfluenza virus types and show high sensitivity and specificity. RT-PCR procedures can either detect single viruses or parainfluenza as a group, with high sensitivity and specificity.

A serological response with detection of a fourfold of greater rise in serum antibody levels between acute and convalescent samples, collected about 3 weeks after the onset of acute illness, may also provides evidence of infection. Antibody can be measured using complement fixation, haemagglutination inhibition or neutralization. Interpretation of a serological test relative to the parainfluenza type producing the infection may be complicated by heterotopic antibody responses seen in some infected individuals.

Respiratory syncytial virus

RSV was first isolated in 1956 from a laboratory chimpanzee with an illness resembling the common cold, and shortly after it was demonstrated to be a human pathogen. Epidemiological studies have shown that RSV represents the single most important cause of serious lower respiratory tract disease, especially bronchiolitis and pneumonia, in infants and children. RSV can cause severe pneumonia and death in persons with underlying immune deficiency.

RSV belongs to the family *Paramyxoviridae* and the genus *Pneumovirus*. It is an enveloped virus with a single-stranded, negative-sense, non-segmented RNA genome with at least 10 viral proteins. Antigenic analysis of RSV has identified two subgroups, A and B, based on their reactivity to a panel of monoclonal antibodies with the B strain further differentiated into two variants, B1 and B2. Annual epidemics of RSV occur during winter in temperate climates and the two subgroups usually co-circulate in the same geographical area, often with a predominance of subgroup A.

Diagnosis of RSV is commonly made by direct examination of respiratory secretions using IF or an EIA technique. A number of commercial rapid point-of-care diagnostic EIA kits which identify RSV antigen in respiratory secretions are now available. These kits provide a high degree of sensitivity or specificity, although they are not as sensitive as DIF. RSV can also be grown in cell cultures but the virus is extremely labile and great care must be taken in transport to ensure that is remains at 4 °C. RSV can also be diagnosed serologically using complement fixation by the detection of a fourfold rise in antibody titre. However, like most respiratory virus serology, this testing is not clinically useful because of the delay in diagnosis. Furthermore, infants may possess maternal antibodies, thus complicating interpretation of serological data.

Adenoviruses

Adenoviruses were isolated from the primary cell cultures of adenoids from children in the early 1950s. Adenoviruses are non-enveloped particles which contain linear double-stranded DNA surrounded by an icosahedral capsid with fibre-like projections extending from each of the 12 capsid vertices.

Adenovirus infections occur worldwide and transmission varies from sporadic to epidemic. Since adenoviruses are very stable they can be easily transmitted in the environment by fomites. Adenoviruses are an important cause of upper and lower respiratory tract disease with types 1, 2, 3, 5 and 7 accounting for about 85% of all infections. Rarely, adenoviruses can be isolated from the lungs, livers, kidneys and brains of patients with fatal infection.

Adenoviruses can be isolated in cell cultures and presumptively recognized by their characteristic cytopathic effect. Currently 52 human adenovirus serotypes have been identified and they can be grouped into six subgenera, designated A through F, based on differing classification schemes concerned with the guanine (G) and cytosine (C) content of their DNA, determined using DNA restriction analysis procedures or PCR.[12] Individual isolates can be serotyped using haemagglutination inhibition, neutralization or IF. However, this is very time consuming and not routinely available. Adenoviruses can be detected directly in respiratory secretions using IF, PCR, ELISA and immunochromatography. Adenovirus infection can be diagnosed serologically using complement fixation by detection of a fourfold or greater rise in antibody titre during convalescence. Type-specific antibody responses can be assayed by haemagglutination inhibition, ELISA and neutralization assay. However, except for epidemiological investigations, antibody determination is a much less efficient means for determining infection than using direct methods such as PCR or antigen detection.

Rhinoviruses

Rhinoviruses are a group of more than 100 serotypes and cause more common cold (minor upper respiratory tract) illness than any other virus that infects the respiratory tract. They account for about one half of common colds occurring in children and consequently cause substantial absenteeism

from schools. In persons with underlying lung disease and immune disorders, rhinoviruses can cause pneumonia.

Rhinoviruses belong to the family Picornaviridae, which are small, non-enveloped viruses with a single-stranded, positive-sense RNA genome. Unlike enteroviruses (also Picornaviridae), rhinoviruses are inactivated when exposed to mild acid (pH <5), accounting for their failure to infect the gastrointestinal tract.

Rhinoviruses also caused otitis media in infants and children, being recovered from middle ear fluid in 10% of subacute or chronic cases that are negative for bacteria. Recent studies associate rhinovirus infection with exacerbations of asthma and with acute lower respiratory tract disease, especially in persons with chronic obstructive airway disease and cystic fibrosis, and those who are immunocompromised. Rhinoviruses prefer to grow at lower temperatures than many other respiratory viruses (33–34 °C), and grow less well at the higher temperature of the lungs than in the nose; however, they can establish infection in the lung.

Type-specific immunity develops following infection and is characterized by IgG neutralizing antibodies in serum and secretions. Long lasting immunity best correlates with the level and secretion of IgA antibody from the nasal mucous membranes.

Diagnosis of rhinovirus infection rarely requires laboratory testing but virus isolation, detection of viral RNA by RT-PCR, antigen detection by DIF in cells from respiratory secretions or detection of a fourfold rise in antibody titres by neutralization test or EIA can be performed if required. Viral isolation in cell culture is very sensitive, especially when collected early in the illness. Viral isolation is labour intensive and requires several days, with confirmation of the individual rhinovirus serotype requiring further antigenic characterization with type-specific antibody. Therefore, it is not used as a routine diagnostic test. RT-PCR is unlikely to be available except as a research procedure.

Coronaviruses

The human coronaviruses 229E and OC43 are an important cause of the common cold and belong to the family *Coronaviridae*, genus *Coronavirus*. They are enveloped with a helical nucleocapsid possessing a single-stranded, positive-sense RNA genome.

Coronavirus infections occur sporadically throughout winter and spring and are distributed worldwide. They are spread by large droplets via the respiratory route. Children commonly experience one coronavirus infection per year, which is about three times more frequently than adults. Coronaviruses are associated with 10–15% of upper respiratory tract illness, mainly causing common colds and otitis media. Pneumonia rarely occurs with non-SARS coronavirus infection.

Type-specific antibodies to single coronavirus types develop in response to infection and persist for about 4 months. Circulating and mucosal antibody confers protection from illness and the possession of coronavirus antibodies increases with age, with circulating antibody found in most children older than 6 years. Nevertheless, re-infections are common.

Laboratory diagnosis of coronavirus infections can be achieved using virus isolation, detection of viral antigen by IF, detection of viral RNA by RT-PCR and/or demonstration of a fourfold or greater rise in antibody titre during convalescence. Virus isolation is complicated and is not generally recommended as a routine diagnostic test since most illnesses are minor common colds. Procedures for antibody measurement include ELISA, neutralization and hemagglutination inhibition.

SARS was first recognized in China in November 2002 and rapidly spread around the world with significant morbidity and mortality, and clusters of affected patients, e.g. healthcare workers and family contacts.[13] A coronavirus was identified as the cause. Interestingly children and adolescents had significantly less severe disease than adults.[14] The SARS coronavirus can be detected using RT-PCR or viral culture. Due to the very high transmissibility of this agent and the severe outcomes, any patient considered to have SARS should be isolated and laboratory specimens collected with high level precautions. The laboratory should be notified as culture of the SARS coronavirus should only be attempted in high biocontainment facilities and specimens may need referral to external public health laboratories.

Human metapneumovirus

Dutch researchers reported the isolation of an agent that induced cytopathic effects on cultured cells, from 28 respiratory specimens collected over a 20 year period.[15] Electron microscopy revealed filamentous viral-like particles, suggesting that the agent was a virus. Using random arbitrarily primed-PCR, the virus discovered was found to be a member of the *Paramyxoviridae* family and the first human pathogen of the genus *Metapneumovirus*; it was named human metapneumovirus (hMPV). Since its initial discovery, hMPV has been identified worldwide. In general, hMPV infection accounts for approximately 2–12% of paediatric lower respiratory illness.[16]

RT-PCR detection is the only reliable method of detection at present. There are no standard methods for isolating hMPV in culture, although this is possible, and monoclonal antibodies to detect the virus by DIF are being tested for clinical utility.

Bocavirus

Human bocavirus is a newly discovered parvovirus that was first identified in Sweden but which occurs globally.[17,18] Bocavirus has been detected in young children with respiratory distress, many with pneumonia with interstitial infil-

trates noted on chest X-ray. However, whether bocavirus is a cause of respiratory disease is yet to be fully determined.

RESPIRATORY ILLNESSES

Common colds

Common colds are very common and are usually self-diagnosed. The term 'cold' does not constitute a single viral entity but rather a syndrome of nasal congestion, sneezing, rhinorrhoea and pharyngitis, with more severe clinical disease associated with high fever in children. Colds are generally self-limiting with a median duration of 9–10 days. Many viruses from a variety of viral families may be associated with this syndrome but the pattern of symptoms associated with colds does not appear to vary significantly between agents. Approximately 40–50% of colds are found to be associated with rhinovirus infection with other frequently occurring agents, including coronaviruses, enteroviruses and non-primary infections with parainfluenza virus and RSV. Transmission of most of the viruses responsible for the common cold is by direct contact and inoculation of virus into the upper respiratory tract. Infection with rhinovirus results in the development of long lasting antibody which is protective against re-infection with the same serotype. This also produces a degree of resistance to challenge with heterologous rhinovirus serotypes but this protection is incomplete and short lived.

Recognized complications of colds include secondary bacterial infection of the paranasal sinuses and/or middle ear and exacerbations of asthma or bronchitis. Viral otitis media commonly complicates colds in children, probably due to eustachian tube dysfunction, with rhinoviruses,[19] RSV, influenza virus and adenovirus[20] detected in the middle ear fluids in 20–40% of cases of otitis media with effusion in children.

Specific viral diagnosis is generally not attempted but if required, isolation of the aetiological agent in cell culture can be performed. RT-PCR may be more sensitive for detection of these agents, especially rhinoviruses, enteroviruses and coronaviruses.

Pharyngitis

Pharyngitis is a very common reason for seeking outpatient medical care. It can be divided into a syndrome with nasal symptoms, which has a predominately viral cause, and cases without nasal symptoms and caused by a diverse spectrum of aetiological agents, including Group A and non-Group A streptococci, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae* and Epstein–Barr virus.

Croup

Croup or viral laryngotracheobronchitis is a clinically distinct illness that predominately affects children under 3 years of age. The characteristic physical finding in croup is inspiratory stridor.

PIVs account for about 75% of cases of croup[21] of which PIV 1 and PIV 2 are the most commonly associated. Other causes of croup include RSV, influenza A and B, rhinoviruses, adenoviruses, as well as *M. pneumoniae*. *M. pneumoniae* and influenza tend to cause croup in older children. PIV 2 and influenza A are associated with more severe disease,[22] but generally the clinical presentation of the croup syndrome due to individual agents is similar. Measles, which is a rare cause of croup, can cause especially severe disease.[23] A specific viral cause for croup can be established with viral culture but is not routinely performed as the clinical syndrome is sufficient for diagnosis and management.

Bronchiolitis

Bronchiolitis is due to inflammation of the bronchioles leading to a clinical syndrome characterised by obstruction of expiratory airflow usually proceeded by nasal congestion and rhinorrhea. There may often be history of exposure to an adult or sibling with a cold or minor respiratory illness or exposure to another infant with bronchiolitis within the day care setting. Mild conjunctivitis may occur in about one third of cases and otitis media is recognized in 5–10%. The hospital course is variable but most infants improve within 3–4 days.[24]

Bronchiolitis is a disease predominately of infancy and the epidemiology of this disease closely parallels the incidence of the major infectious cause, RSV. The peak incident age is in infants between 2 and 6 months with over 80% of cases occurring in the first year of life.[25]

RSV causes the majority of cases of bronchiolitis however other respiratory viruses include PIVs, influenza and rhinoviruses. PIV 3 and adenovirus types 3, 7 and 21 are relatively uncommon but may be associated with more severe disease including bronchiolitis obliterans.[26] Rhinoviruses can cause bronchiolitis in infants with bronchopulmonary dysplasia.[26–28] Pertussis is occasionally confused with bronchiolitis. Anatomic defects such as vascular rings, foreign bodies and gastro-oesophageal reflux are additional differential diagnoses.

Viral pneumonia (pneumonitis)

Bronchiolitis and pneumonia are both part of the spectrum of lower respiratory tract involvement, frequently co-exist and cannot be clearly distinguished. Pneumonia is defined by the development of abnormalities in alveolar gas exchange accompanied by inflammation of the lung parenchyma, often associated with visible changes on chest X-ray, CT scanning or gallium scanning. There can be considerable variety to the presentation of viral pneumonia depending on the age and immunological competence of the host, as well as the specific viral pathogen. Viral

pneumonia is an important cause of morbidity and mortality in individuals with a compromised immune system.

The clinical presentation varies considerably with the specific causative agent but typically includes fever and lower respiratory tract symptoms and signs, such as tachypnoea, non-productive cough, wheeze and increased breath sound. Very young infants may present with apnoeic episodes with minimal fever. Bacterial superinfection is a potential and serious complication of viral lower respiratory tract infection, particularly with influenza. Underlying cardiopulmonary disease such as valvular heart disease or chronic obstructive pulmonary disease are well recognized risk factors for increased severity of disease with viral pneumonia.

Diagnosis of the specific cause of an acute pneumonia due to a particular viral agent is complicated by difficulty in obtaining appropriate lower respiratory tract samples for culture and in isolating or detecting certain pathogens, and additionally by the frequent asymptomatic shedding of some viruses, e.g. herpes simplex virus or adenoviruses. While viruses are clearly important and frequent causes of pneumonia in young children, they are less apparent in older children. RSV has been associated with the largest proportion of viral pneumonia in young children, particularly if accompanied by bronchiolitis.[29] PIV 3 and influenza A and B are significant causes of pneumonia in children, especially during periods of epidemic prevalence.[30] Adenoviruses are frequently isolated from children with respiratory disease and are implicated in about 10% of childhood pneumonias. However, their true impact is difficult to access because of the long intermittent asymptomatic respiratory shedding of adenoviruses in children. Adenoviruses have been described as cause of significant outbreaks of viral pneumonia in institutionalized children. Pneumonia is the most frequent serious complication of measles. Rhinoviruses have been associated with community-acquired pneumonia in children despite their apparent temperature sensitivity. Other occasional causes are enteroviruses, HSV and VSV.

Serological diagnosis essentially establishes a temporal but not a causal relationship between a viral infection and a clinical syndrome, and a positive serological result may be misleading during times of high prevalence of a particular viral agent.

REFERENCES

1. Fenner FJ, White DO. Laboratory diagnosis of viral disease. *Medical Virology*. 4th edition. San Diego: Academic Press; pp. 191–218.
2. Forman MS, Valsamakis A. Specimen collection, transport and processing: Virology. In: Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA, eds: *Manual of Clinical Microbiology*. 9th edition. Washington DC: ASM Press; pp. 1284–1296.
3. Dunn JJ, Woolstenhulme RD, Langer J, Carroll KC. Sensitivity of respiratory virus culture when screening with R-Mix fresh cells. *J Clin Microbiol* 2004; **42**: 79–82.
4. Olivo PD. Transgenic cell lines for detection of animal viruses. *Clin Microbiol Rev* 1996; **9**: 321–334.
5. Patel N, Kauffmann L, Baniewicz G, Forman M, Evans M, Scholl D. Confirmation of low-titer, herpes simplex virus-positive specimen results by the enzyme-linked virus-inducible system (ELVIS) using PCR and repeat testing. *J Clin Microbiol* 1999; **37**: 3986–3989.
6. Schutzbank TE, McGuire R. Immunofluorescence. In: Specter S, Hodinka RL, Young SA, eds: *Clinical Virology Manual*. 3rd edition. Washington DC: ASM Press; pp. 69–78.
7. Mackie PL, Joannidis PA, Beattie J. Evaluation of an acute point-of-care system screening for respiratory syncytial virus infection. *J Hosp Infect* 2001; **48**: 66–71.
8. Cazacu AC, Greer J, Taherivand M, Demmler GJ. Comparison of lateral-flow immunoassay and enzyme immunoassay with viral culture for rapid detection of influenza virus in nasal wash specimens from children. *J Clin Microbiol* 2003; **41**: 2132–2134.
9. Leland DS, Ginocchio CC. Role of cell culture for virus detection in the age of technology. *Clin Microbiol Rev* 2007; **20**: 49–78.
10. Beigel JH, Farrar J, Han AM et al. Writing Committee of the World Health Organization (WHO). Consultation on Human Influenza A/ H5. Avian influenza (H5N1) infections in humans. *N Engl J Med* 2005; **353**: 1374–1385.
11. Yuen KY, Chan PK, Peiris M et al. Clinical features and rapid viral diagnosis of human disease associated with avian influenza A H5N1 virus. *Lancet* 1998; **351**: 467–471.
12. Wadell G. Adenoviruses. *Curr Top Microbiol Immunol* 1984; **110**: 191–220.
13. Peiris JSM, Lai ST, Poon LLM et al. Coronavirus as a possible cause of severe acute respiratory syndrome. *Lancet* 2003; **361**: 1319–1325.
14. Hon KLE, Leung CW, Cheng WTF et al. Clinical presentations and outcome of severe acute respiratory syndrome in children. Research letters. *Lancet* 2003; **361**: 1701–1703.
15. van den Hoogen BG, de Jong JC, Groen J et al. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nature Med* 2001; **7**: 719–724.
16. Alto WA. Human metapneumovirus: a newly described respiratory tract pathogen. *J Am Board Fam Pract* 2004; **17**: 466–469.
17. Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B. Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proc Natl Acad Sci USA* 2005; **102**: 12891–12896.
18. Foulongne V, Rodiere M, Segondy M. Human bocavirus in children. *Emerg Infect Dis* 2006; **12**: 862–863.
19. Pikraranta A, Virolainen A, Jero J, Arruda E, Hayden FG. Detection of rhinovirus, respiratory syncytial virus and coronavirus infections in acute otitis media by reverse transcription polymerase chain reaction. *Pediatrics* 1998; **102**: 291–295.
20. Henderson FW, Collier AM, Sanyal MA et al. A longitudinal study of respiratory viruses and bacteria in the etiology of acute otitis media with effusion. *N Engl J Med* 1982; **306**: 1377–1383.
21. Denny FW, Murphy TF, Clyde WAJ, Collier AM, Henderson FW. Croup: an 11-year study in a pediatric practice. *Pediatrics* 1983; **71**: 871–876.
22. Kim H, Brandt CD, Arrobio JO, Murphy B, Chanock RM, Parrott RH. Influenza A and B virus infection in infants and young children during the years 1957–1976. *Am J Epidemiol* 1979; **109**: 464–479.
23. Ross LA, Mason WH, Lanson J, Deakers TW, Newth CJL. Laryngo-tracheo-bronchitis as a complication of measles during an urban epidemic. *J Pediatr* 1992; **121**: 511–515.
24. Hall CB, Hall WJ, Speers DM. Clinical and physiological manifestations of bronchiolitis and pneumonia: outcome of respiratory syncytial virus. *Am J Dis Child* 1979; **133**: 798–802.
25. Parrott RH, Kim HW, Arrobio JO. Epidemiology of respiratory syncytial virus infection in Washington, DC. *Am J Epidemiol* 1973; **98**: 289–300.
26. Becroft DMO. Brochiolitis obliterans, bronchiolitis, and other sequelae of adenovirus type 21 infection in young children. *J Clin Pathol* 1971; **24**: 72–81.

27. McMillan JA, Weiner LB, Higgins AM, MacKnight K. Rhinovirus infection associated with serious illness among pediatric patients. *Pediatr Infect Dis J* 1993; **12**: 321–325.

28. Wright AL, Taussig LM, Ray CG, Harrison HR, Holberg CJ. The Tucson children's respiratory study: II. Lower respiratory tract illness in the first year of life. *Am J Epidemiol* 1989; **129**: 1232–1236.

29. Chidekel AS, Bazzy AR, Rosen CL. Rhinovirus infection associated with severe lower respiratory tract illness and worsening lung disease in infants with bronchopulmonary dysplasia. *Pediatr Pulmonol* 1994; **18**: 261–263.

30. Sugaya N, Nerome K, Ishida M *et al.* Impact of influenza virus infection as a cause of pediatric hospitalization. *J Infect Dis* 1992; **165**: 373–375.