



Respiratory Virus Screen

Introduction

Most acute respiratory tract infections are caused by a number of commonly encountered viruses. There are considerable overlaps among the symptoms caused by different respiratory viruses and they cannot be consistently distinguished by clinical presentation alone. Multiplex reverse transcriptase (RT-) PCR screening for common respiratory viruses represents an excellent method of rapidly identifying the cause of infection. Respiratory screening may be of great benefit both in ensuring optimal patient treatment and in deciding whether to implement measures to control the spread of infection (Caliendo, 2011). Early diagnosis can help clinicians to minimise the use of inappropriate treatments that may be costly and/or have potentially harmful side effects. Screening for respiratory viruses may be particularly valuable when dealing with immunocompromised patients among whom many common viral infections carry a relatively high morbidity/mortality rate (Choi *et al.*, 2013, Shah *et al.*, 2012).

Micropathology Ltd offers a real-time (RT-) PCR screen for a broad range of respiratory viruses. These include influenza viruses A and B, adenovirus, metapneumovirus (MPV), respiratory syncytial virus (RSV), rhinovirus and parainfluenza viruses 1-4. Our extended panel additionally includes coronaviruses 229E, OC43, NL63 and HKU1. In many cases we are able to provide further subtyping where a virus is detected, subject to the viral load being sufficient to perform sequencing (see below). A separate assay for bocavirus is available upon request. Besides viral pathogens we are able to perform testing for a number of prokaryotic and eukaryotic organisms including *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *Mycobacterium tuberculosis*, *Pneumocystis jiroveci*, *Chlamydia pneumoniae* and *Chlamydia psittici*. Please visit our website for a complete list of all our assays.

Beside its diagnostic value, the respiratory screen can be readily applied to very large numbers of samples within a research project (see for example, Herberg *et al.*, 2013). Our experience has confirmed that we are able to generate large amounts of data for publication both quickly and cost-effectively using this approach. There is evidence from this sensitive screen that some children are found to carry multiple viruses and this is of particular interest when interpreting the nature of the pathology. Please contact Dr. Edward Sumner at Micropathology Ltd. if you would like to discuss using the respiratory screen as part of a research project (email: e.sumner@micropathology.com).

Influenza A and B

Timely diagnosis of influenza viruses may be critical for immunocompromised patients because of the severity of illness caused by these viruses. Unlike most respiratory viruses effective vaccines and antiviral therapies are available to help limit the spread of infection (Santesso *et al.*, 2013). The latter are chiefly neuraminidase inhibitors, which interfere with virus cell entry and release. Neuraminidase inhibitors include oseltamivir (which is marketed under the trade name “Tamiflu”). Our assay distinguishes between influenza types A and B, and if required we are usually able to perform typing to determine whether type A/H1N1 pandemic strain is present or not.

MPV and RSV

MPV and RSV are assumed to cause relatively mild respiratory tract infection but they may cause more severe clinical disease in the very young, those with underlying lung disease, the elderly and the immunocompromised. RSV is commonly associated with croup in infants. Severe infection with RSV is one of the leading causes of hospitalisation among very young children and is an important cause of paediatric mortality (Kusel *et al.*, 2005). MPV was discovered relatively recently and is often considered to cause symptoms similar to those of RSV. Treatment for both viruses is largely through supportive therapy, although treatment for RSV with the antiviral drug ribavirin is sometimes initiated (Gueller *et al.*, 2013).

Parainfluenza viruses 1-4

Parainfluenza viruses are members of the paramyxovirus family. In common with many other respiratory viruses they are responsible for a broad spectrum of symptoms including rhinorrhea, cough, bronchiolitis and pneumonia. They are particularly important in young children among whom they are one of the most common causes of croup and/or hospitalisation after RSV (Leung *et al.*, 2004). The overwhelming majority of children show serological evidence of parainfluenza infection by age six (van der Logt *et al.*, 1982). All four parainfluenza viruses cause a full spectrum of respiratory symptoms, however croup is most commonly associated with type 1, while bronchiolitis and pneumonia are most often associated with types 1 and 2 (Henrickson, 2003). Our assay distinguishes between all four parainfluenza types.

Rhinoviruses

Rhinoviruses are thought to be responsible for over half of all viral respiratory tract infections. There are three rhinovirus species termed human rhinovirus- (HRV-)A, B and C. Although rhinoviruses have been studied extensively for several decades HRV-C was only recently recognised as a distinct species (Lau *et al.*, 2007). This is due in part to the difficulty of isolating members of the HRV-C species by virus culture. There is evidence that infection with HRV-C may cause particularly severe symptoms (Lauinger *et al.*, 2013). Infection with HRV-C during early childhood *may* increase the likelihood of the onset of asthma (Gern, 2010). The multiplex screen detects all three rhinovirus species and if required we are usually able to perform a separate assay to determine genotype.

Coronaviruses

Most coronaviruses are either difficult or impossible to culture and so molecular methods are particularly important for detecting and studying these pathogens (Pyrce *et al.*, 2010). Our assay can detect and distinguish between coronaviruses 229E, OC43, NL63 and HKU1, which together are responsible for several per cent of acute respiratory tract infections among both paediatric and adult populations (Prill *et al.*, 2012, Woo *et al.*, 2012). Differing patterns of seasonal infection rates have been observed among different coronavirus types; co-infections with other viruses are particularly prevalent (Gaunt *et al.*, 2010).

Please note that the assay is not designed to detect either severe acute respiratory syndrome (SARS) coronavirus or Middle East respiratory syndrome (MERS) coronavirus (the high level of containment required for dealing with these pathogens precludes us from including these viruses in the multiplex screen).

Adenovirus

Adenoviruses are common causes of human disease including conjunctivitis, gastroenteritis and respiratory tract infections. Respiratory manifestations include pharyngitis, rhinorrhea, fever, bronchitis and pneumonia. Adenoviruses are one of several causes of acute respiratory disease syndrome (ARDS), a relatively rare but life threatening lung condition in which impaired gas exchange causes hypoxemia that may lead to multiple organ failure (Lai *et al.*, 2013). Left untreated ARDS is frequently fatal. Adenoviruses are of particular concern among hematopoietic stem cell transplant patients, in whom they may cause a disseminated multi-organ infection and/or a particularly high rate of morbidity and mortality (Neofytos *et al.*, 2007).

There are more than 50 adenovirus serotypes assigned to six species, A to F. Certain serotypes are associated with differing patterns of disease severity or clinical presentation (although there are considerable overlaps among the presentations caused by differing serotypes) (Lynch *et al.*, 2011). We are able to perform genotyping if required.

Summary

Our respiratory screen provides a sound method of identifying the cause of viral respiratory tract infection. We are always happy to discuss tailoring the screen to suit clinical need. Assay performance is subject to regular external quality assurance programmes and oligonucleotide sequences are reviewed frequently to ensure that any newly emerging virus strains will be detectable. The screen has proven to be highly useful as a research tool for high-throughput sample analysis and together with our genotyping assays, can be used to provide a wealth of epidemiological data.

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