



Respiratory Virus Screen

Introduction

Most acute respiratory tract infections are caused by commonly encountered viruses. As respiratory viruses cause a number of overlapping signs and symptoms it is difficult to determine the causative agent by clinical presentation alone.

Traditionally we have used Multiplex reverse transcriptase (RT-) PCR as a means of rapidly screening for respiratory pathogens. Screening may be of great benefit both in guiding optimal patient treatment and in deciding whether to implement measures to control the spread of infection (*Caliendo, 2011*).

We have now advanced our assay portfolio to account for increased numbers of samples and in line with the latest nucleic acid technology. Luminex® is a molecular platform for respiratory screening. The Luminex® respiratory pathogen panel (RPP) assay allows us to offer comprehensive respiratory screening for up to 19 viral targets. The assay has undergone extensive in-house validation in accordance with our UKAS laboratory accreditation and is suitable for a variety of sample types (see below). An outstanding feature is the minimal volume of sample required for a significant diagnostic yield (>200ul).

PATHOGEN TARGET

Adenovirus

Influenza A

Influenza A H1

Influenza A H3

Influenza B

Parainfluenza type 1-4

RSVA

RSVB

Rhinovirus

Enterovirus

VALIDATED SAMPLE TYPES*

Nasopharyngeal swabs

Throat swabs

Bronchoalveolar lavage (BALS)

Nasal aspirates

Tracheal aspirates

Sputum

Coronaviruses:
229E/OC43/NL63/HKU1
Human Metapneumovirus
Human Bocavirus

*Represents those sample types validated for RPP assay use by Luminex®. Other sample types have undergone in house evaluation and are available to be tested on request.

A broad coverage of respiratory viruses in a single test can be of great benefit both in ensuring optimal patient treatment and in deciding whether to implement measures to control the spread of infection. Early diagnosis can help clinicians avoid the use of inappropriate treatments that may be costly and/or have potentially harmful side effects. This is 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*).

When viruses are detected by the Luminex RPP assay, often we are able to provide further subtyping of the pathogen, subject to the viral load being sufficient to perform sequencing. In addition, we offer diagnostic testing for a number of prokaryotic and eukaryotic organisms through our established RT-PCR assays, including *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *Mycobacterium tuberculosis* and *Pneumocystis jiroveci*. Please visit our website for a complete list of all our assays.

The diagnostic power of the Luminex RPP screen is not only applicable to our diagnostic services, but can also be used for high-throughput processing of research samples (*Herberg et al., publication pending 2018*). The sensitivity of the RPP screen allows us to detect multiple viruses per sample, which can be of particular interest when interpreting a patient's clinical presentation and exposure. We find that we are able to generate large amounts of data for research projects both quickly and cost-effectively using this approach, which is particularly advantageous in studies involving large cohorts. Please contact Dr. Edward Sumner, Dr Marie Voice and Miss Balraj Johal at Micropathology Ltd. if you would like to discuss using the screen as part of your research project (email: e.sumner@micropathology.com, m.voice@micropathology.com and b.johal@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 targeting influenza are available (*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”). The Luminex® RPP assay distinguishes between influenza types A and B and can determine whether the types A/H1N1 and A/H3N2 pandemic strains are present or not.

MPV and RSV

MPV and RSV are usually thought to cause relatively mild respiratory tract infection but this may be more severe in the very young, 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 childhood mortality (*Kusel et al., 2005*). There is also an association between RSV and the development of pneumococcal pneumonia (*Weinberger et al., 2015*). MPV was discovered relatively recently and causes 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*). The Luminex® RPP assay can distinguish between all four parainfluenza types.

Rhinoviruses & Enteroviruses

Both Rhinoviruses and Enteroviruses are part of the Picornaviridae family and are within the Enterovirus genus. There are three rhinovirus species termed human rhinovirus- (HRV-) A, B and C and are thought to be responsible for over half of all viral respiratory tract infections. Although rhinoviruses have been studied extensively for several decades HRV-C was only recently recognised as a distinct species. This is due in part to the difficulty of isolating HRV-C species by virus culture. There is evidence that infection with type C may cause particularly severe symptoms. Infection during early childhood may increase the likelihood of the onset of asthma. All three rhinovirus species are detected by the RPP screen and if required we are able to perform a separate assay to determine genotype.

The four Non-rhinovirus enterovirus family members classified as EV-A, EV-B, EV-C & EV-D. Enteroviruses have a diverse tropism and can cause a wide range of clinical syndromes including myocarditis, meningitis and paralysis. However, some enterovirus subtypes (notably EV-C and EV-D) are associated with the respiratory system and cause rhinovirus-like symptoms. Although typically mild such symptoms can lead to complications such as wheezing, breathing difficulties and pneumonias. In 2014 an EV-D68 was identified as the cause of a notable outbreak of respiratory disease in children and has been linked to neurological complications. The incidence of EV-D68 infections continue to rise, which can be attributed to both a mutation-driven increase in virulence and more accurate identification (*reviewed by Royston & Tapparel, 2016*). Our PCR assays allow us to distinguish between rhinovirus and enterovirus family members and we can perform genotyping on request.

Coronaviruses

Most coronaviruses are either difficult or impossible to culture and so molecular methods are particularly important for detecting and studying these pathogens. The RPP screen can 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*). Human coronaviruses display differing patterns of seasonal infection rates and co-infections with other viruses are common (*Gaunt et al., 2010*). 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 accepting specimens with suspected SARS or MERS).

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. Left untreated ARDS is frequently fatal. Adenoviruses are of particular concern among hematopoietic stem cell transplant patients, in whom they may cause a disseminated infection and/or a high rate of morbidity and mortality.

There are 56 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). We are able to perform genotyping on samples if required.

Bocavirus

Human Bocavirus is a member of the parvoviridae family and was first identified in 2005. Human Bocavirus 1 (HBoV1) is associated with respiratory symptoms and is frequently detected in the airways of children under 5yrs who present with respiratory illnesses. Understanding the pathogenicity of HBoV1 is complex as it is typically co-associates with other respiratory viruses. Although its etiology in respiratory disease is under defined, there is evidence that HBoV1 has a causative role in acute wheezing (*Yu Deng et al, 2012*) and can exacerbate respiratory illnesses (*T del Rossal et al, 2016*). Rarely, life-threatening incidence of HBoV1 infection have been reported in infants (*T. Ursic et al, 2011*).

Summary

Our use of traditional PCR together with Luminex® provides an effective method of identifying the cause of respiratory tract infection and we are always happy to discuss tailoring the screen to suit clinical need. Assay performance is subject to regular external quality assurance programmes in line with our UKAS laboratory accreditation. 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.