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Respiratory 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 Polymerase Chain Reaction (PCR) as a means of rapidly screening for respiratory pathogens, as screening is of great benefit both in guiding optimal patient treatment and in deciding whether to implement measures to control the spread of infection *(Sze Hwei Lee et al., 2019).*

We have now advanced our assay portfolio to account for an increased number of patient samples, and to ensure our assays are in-line with the latest nucleic acid technology. The NxTAG Luminex® Respiratory Pathogen Panel (RPP) assay allows us to offer a comprehensive respiratory screen for up to 19 viral and 3 bacterial targets (see below), which are vital in the diagnosis of respiratory illness. The RPP assay is performed via the Luminex® MAGPIX, a microfluidics-based molecular platform.

The RPP assay kit has undergone extensive in-house verification 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 (>200µl).

VERIFIED SAMPLE TYPES*

- Nasopharyngeal swabs
- Throat swabs
- Bronchoalveolar lavage (BALS)
- Nasal aspirates
- Tracheal aspirates
- Sputum

***Represents patient sample types verified in-house for use in the Luminex® RPP assay. Other sample types may be tested upon request.**

VIRAL PATHOGEN TARGETS

- Adenovirus
- Influenza A H1, Influenza A H3
- Influenza B
- Parainfluenza type 1-4
- Respiratory syncytial virus A (RSVA), Respiratory syncytial virus B (RSVB)
- Rhinovirus
- Enterovirus
- Seasonal Coronaviruses: 229E/OC43/NL63/HKU1
- Human Metapneumovirus (MPV)
- Human Bocavirus

BACTERIAL PATHOGEN TARGETS

- *Legionella pneumophila*
- *Mycoplasma pneumoniae*
- *Chlamydia pneumoniae*

Pathogens detected by the Luminex ® Respiratory Pathogen Panel (RPP).

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 respiratory infections carry a high morbidity and mortality *(Andrea Cortegiani et al., 2018).*

When viruses are detected by the Luminex RPP assay, we are often able to provide further subtyping of the pathogen, such as adenovirus and enterovirus (subject to sufficient viral load). 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 *(Poornima Ramanan et al., 2017).* 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 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 us if you would like to discuss using the screen as part of your research project (email: info@micropathology.com).

Influenza A and B

Influenza A and B are classified within the *Orthomyxoviridae* family, and target the respiratory tract within human hosts. Upon infection, timely diagnosis of influenza viruses may be critical for immunocompromised patients, due to the severity of illness caused by these viruses. Unlike most respiratory viruses, effective vaccines and antiviral therapies targeting influenza are available. The latter are chiefly neuraminidase inhibitors which include oseltamivir (marketed under “Tamiflu”), that interfere with viral cell entry/release *(Xiao-Guang Li, 2022).* The Luminex® RPP assay is able to differentiate between influenza types A and B, and distinguish between A subtypes H1N1 and H3N2.

Metapneumovirus (MPV) and Respiratory Syncytial Virus (RSV)

MPV and RSV fall within the *Pneumoviridae* family, and are primarily thought to cause relatively mild respiratory tract infections. However, in young, elderly, and immunocompromised populations they may cause severe symptoms, resulting in hospitalisation *(Leigh M Howard, 2021)*. RSV is commonly associated with croup in infants, and is well recognised as a significant cause of childhood mortality *(Marco Del Riccio et al., 2023)*. Moreover, the association between RSV and the development of pneumococcal pneumonia can result in poor respiratory sequelae, highlighting the importance of RSV diagnosis and treatment *(Tom Wilkinson et al., 2023)*. Similarly, MPV is believed to causes symptoms comparable to those of RSV, and has been well-defined to cause ARDS within an ICU setting (*Loreto Vidaur, 2019)*. It is therefore imporant to diagnose both RSV and MPV to mitigate further transmission. The Luminex® RPP assay is able to distinguish between RSV and MPV infection, and further differentiate between RSV type A (RSVA) and RSV type B (RSVB).

Parainfluenza viruses (HPIV) 1- 4

Parainfluenza viruses (1-4) are members of the *Paramyxovirus* family, and are responsible for a range of symptoms, including rhinorrhoea, tussis, bronchiolitis and pneumonia *(Akhil Chellapuri, 2022).* They are particularly important within young children, among whom they are one of the most common causes of croup and/or hospitalisation following RSV infection *(Ji Yoon Han, 2022).* 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 *(Akhil Chellapuri et al., 2022)*. The Luminex® RPP assay is able to distinguish between all four parainfluenza types (HPIV types 1-4).

Rhinoviruses (HRV) & Enteroviruses (EV)

Both rhinoviruses and enteroviruses are part of the *Picornaviridae* family, *Enterovirus* genus. There are three observed rhinoviruses species, termed human rhinovirus (HRV) A, B and C, responsible for over half of all viral respiratory tract infections *(Aripuana Watanabe, 2010)*. While rhinovirus infection is often mild, infection during early childhood has been extensively linked to asthma onset, as well as asthma exacerbation *(Yu-Tsun Su, 2020, Haiwen Liu, 2020*). There are 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 respiratory system infection and can cause rhinovirus-like symptoms. Although typically mild, such symptoms can lead to wheezing, dyspnoea and pneumonia. In 2014, EV-D68 was identified of as the cause of a notable outbreak of respiratory disease in children, linked to neurological tropism and hospitalisation. Currently, the incidence of EV-D68 infections continue to rise, attributed to both a mutation-driven increase in virulence, as well as advancements in molecular diagnostics and accurate identification *(Léna Royston, 2016)*. Our in-house PCR assays allow us to distinguish between rhinovirus and enterovirus, and we are able to perform genotyping upon request.

Coronaviruses

Pandemic (SARS-Cov-2)

Micropathology Ltd offers an in-house assay for the detection of SARS CoV-2 RNA, which is available upon request, and is routinely performed when the viral respiratory screen is requested.

Seasonal (HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1)

Seasonal coronaviruses belong to the *Coronaviridae* family, with notable species including HCoV-229E, OC43, NL63 and HKU1, and are responsible for the infection of the upper respiratory tract. Symptoms of seasonal coronavirus often include coryza, pyrexia and dyspnoea, and while most infection is self-limiting, hospitalisation may be necessary *(Taehee Kim, 2021, Zhi-Qi Zeng, 2017)*. Due to the fastidious nature of coronaviruses, identification is primarily achieved using molecular methods such as the Luminex RPP screen, which can distinguish between HCoV-229E, OC43, NL63 and HKU1 with a high degree of both accuracy and sensitivity.

Please also note that this assay is not designed to detect Middle East respiratory syndrome coronavirus (MERS-CoV), due to the high level of containment required.

Adenovirus (AdV)*

Adenoviruses are classified within the *Adenoviridae* family, organised into 56 adenovirus serotypes and assigned to six species A to F, with certain serotypes associated with differing patterns of disease severity or clinical presentation. AdV is commonly associated with conjunctivitis, gastroenteritis and respiratory tract infections within humans, with respiratory manifestations including pharyngitis, rhinorrhoea, pyrexia, bronchitis and pneumonia *(Xinye Wang, 2021)*. While AdV is one of several viral causes of acute respiratory disease syndrome (ARDS), infection may develop and impair alveolar gas exchange which, left untreated and undiagnosed, is frequently fatal *(Xuefei Chen, 2020).* Furthermore, Adenoviruses are of particular concern among hematopoietic stem cell transplant patients, in whom AdV may disseminate and increase both morbidity and mortality rates *(Marco W. Schilham, 2002)*. Following Adenovirus detection, we are able to perform genotyping upon request.

Bocavirus (HBoV)

Human Bocavirus is a member of the *Parvoviridae* family and was first identified in 2005. There are four recognised human Bocaviruses (HBoV 1-4), however HBoV1 has been associated with respiratory symptoms, and is frequently detected in the airways of children under 5yrs presenting with respiratory illnesses. However, understanding the pathogenicity of HBoV1 is complex as it typically co-associates with other respiratory viruses. Although there is a paucity of information, evidence suggests that HBoV1 has a causative role in acute wheezing and exacerbation of respiratory illnesses *(Yu Deng, 2012)*. Rarely, life-threatening incidence of HBoV1 infection have been reported in infants *(Tina Uršič, 2012).* The Luminex® RPP assay is able to detect respiratory bocavirus (HBoV1-4).

Legionella pneumophila (L. pneumophila, Lpn)

Legionella pneumophila is a Gram-negative, facultative intracellular bacterium that targets and infects the lower respiratory tract, resulting in atypical pneumonia. While there are more than 58 distinct species of *Legionella spp*., only 25 are known to be harmful to humans. The most prominent of the *Legionella* species is *L. pneumophila*, accounting for more than 95% of all *Legionella* cases *(James T. Walker, 2021).* The Luminex RPP panel detects only *Legionella pneumophila* within respiratory samples. However, since these organisms are notifiable to UKHSA, they will be appropriately caveated on the patient report as: ‘Please note, Legionella spp. appears as a ‘notifiable organism’ causative agent on the UKGOV website and should be notified to relevant Public Health Authority’.

Mycoplasma pneumoniae (M. pneumoniae, Mpn)

Mycoplasma pneumoniae is a Gram-negative bacterium, categorised into two major groups: P1 type 1 (P1-1) or P1 type 2 (P1-2), depending on the P1 protein genotype present. Infection with either type of *M. pneumoniae* is often mild and self-limiting, however patients can develop severe and fulminant disease without diagnosis and treatment *(Jasna Rodman Berlot, 2021).* Furthermore, since *M. pneumoniae* is a common cause of bacterial atypical pneumonia, rapid diagnosis and treatment will mitigate further clinical sequelae and transmission. The Luminex ® RPP respiratory screen is able to detect *M. pneumoniae*.

Please also note that Micropathology Ltd offers an in-house PCR assay for the detection of *Mycoplasma pneumoniae,* which maintans a superior sensitivity to the respiratory screen.

Chlamydia Pneumoniae (C. pneumoniae, Cpn)

Chlamydia pneumoniae, formally known as *Chlamydophila pneumoniae* (from 1999 to 2015), is a Gram-negative bacterium which targets and infects the upper respiratory tract within human hosts. *C. pneumoniae* primarily causes atypical pneumonia, with symptoms ranging from asymptomatic or mild to severe pneumonia, which can result in related complications such as asthma exacerbation, encephalitis and cardiovascular dysfunction *(Doriane Calmes, 2021, Hadeel A. Nasser, 2019).* For this reason, it is clinically important to pursue a diagnosis and treatment for *C. pneumoniae*. The Luminex ® RPP respiratory screen is able to detect *Chlamydia pneumoniae*.

Please also note that Micropathology Ltd offers an in-house PCR assay for the detection of *Chlamydia pneumoniae,* which maintans a superior sensitivity to the respiratory screen.

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 internal quality testing and external quality assurance programmes in line with our UKAS laboratory accreditation. The respiratory 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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