Pathology (April 2015) **47(3)**, pp. 243–249

Molecular diagnosis of respiratory viruses

LUCY K. SOMERVILLE[1], V. MALA RATNAMOHAN[1], DOMINIC E. DWYER[1,2,3] AND JEN KOK[1,2,3]

[1]Centre for Infectious Diseases and Microbiology Laboratory Services, Institute of Clinical Pathology and Medical Research, Westmead Hospital, Westmead, [2]Marie Bashir Institute for Infectious Diseases and Biosecurity, University of Sydney, Westmead Hospital, Westmead, and [3]Centre for Research Excellence in Critical Infections, University of Sydney, Westmead Hospital, Westmead, NSW, Australia

Summary

The increasing availability of nucleic acid amplification tests since the 1980s has revolutionised our understanding of the pathogenesis, epidemiology, clinical and laboratory aspects of known and novel viral respiratory pathogens. High-throughput, multiplex polymerase chain reaction is the most commonly used qualitative detection method, but utilisation of newer techniques such as next-generation sequencing will become more common following significant cost reductions. Rapid and readily accessible isothermal amplification platforms have also allowed molecular diagnostics to be used in a 'point-of-care' format. This review focuses on the current applications and limitations of molecular diagnosis for respiratory viruses.

Key words: Diagnostics, molecular, PCR, respiratory viruses.

Received 26 January, revised 3 February, accepted 5 February 2015

INTRODUCTION

Worldwide, viral respiratory tract infections cause significant morbidity and mortality. In the United States of America (USA), pneumonia and influenza ranks sixth as the cause of hospitalisation among Medicare beneficiaries.[1] In New South Wales, Australia, influenza and pneumonia was responsible for 9.1% of total deaths in 2013.[2] However, these are likely underestimates of the true burden of influenza as unrecognised infection may result in respiratory or cardiovascular hospitalisations and deaths.[3–5] Similarly, other respiratory viruses may also cause severe respiratory infections, particularly in the young, elderly or immunocompromised.[6,7]

Laboratory confirmation of the aetiology of viral respiratory tract infection guides treatment, obviates the need for further unnecessary testing and is useful for epidemiological purposes, including planning vaccination strategies. When selecting the most appropriate test, clinicians should consider the availability, performance and turnaround times of the different diagnostic methods. Although viral culture remains the 'gold standard' for diagnosis, nucleic acid amplification tests (NAATs) are predominantly used given their increased sensitivity, specificity, breadth, and reduced turnaround time to pathogen detection.[8] NAATs can also be used for typing, subtyping, quantitation of viral loads and detection of antiviral resistance. Nevertheless, NAATs are not perfect, and their role in the laboratory diagnosis of respiratory viruses is constantly evolving.

Herein, we discuss the applications, advantages and limitations of NAATs in the diagnoses and clinical management of respiratory viruses.

THE ROLE OF NUCLEIC ACID AMPLIFICATION TESTING

Qualitative detection and quantitation of respiratory viruses

NAATs are primarily used to determine the respiratory viruses responsible for infection, as the viral aetiology is unlikely to be reliably distinguished on clinical features alone. Table 1 outlines the respiratory viruses that are commonly detected using NAATs.

Although not routinely performed, quantitative detection may provide useful information on the severity and prognosis of viral respiratory infections, efficacy and resistance development during antiviral therapy and the duration of viral shedding to inform infection control measures.[9–11] In hospitalised adults with influenza infection, viral RNA detection served as a surrogate for persistent isolation of virus, thus enabling the identification of risk factors for severe infection.[11]

The pathogenesis of novel or emerging respiratory viruses may be guided by studying viral replication; for example, over time, in different patient groups (adult or paediatric, immunocompetent or immunosuppressed), in relation to symptomatology, in response to treatment, in different tissues, and in different locations of the respiratory tract.[12–14] In the paediatric population, quantitation of respiratory viruses may be used to differentiate clinically significant infection versus asymptomatic infection or 'carriage'.[9,15] Quantitation is also useful to understand the clinical impact of co-infections, including the pathogenicity of frequently detected viruses such as human bocavirus (HBoV) and polyomaviruses. The duration of antiviral therapy for influenza virus infections may also be optimised with viral load measurements.[16] However, accurate quantitation of viral load may be compromised by the non-uniformity in sample volumes, as in samples like nose and throat swabs (NTS).

Detection of antiviral resistance

Although neuraminidase inhibitors (NIs) are widely prescribed for influenza infections, a number of antivirals are currently in phase II or III trials for non-influenza viruses.[17] Detection of antiviral resistance is best described for NIs, with near 100% resistance of seasonal influenza A/H1N1 strains to oseltamivir prior to the influenza pandemic of 2009. This influenza subtype

Print ISSN 0031-3025/Online ISSN 1465-3931
DOI: 10.1097/PAT.0000000000000240

Table 1 Respiratory viruses detected by nucleic acid amplification tests

Respiratory virus	Virology	Diagnostic methods
Influenza virus	ssRNA (−)	RT-PCR LAMP
Respiratory syncytial virus (RSV)	ssRNA (−)	RT-PCR LAMP
Human rhinovirus (HRV)	ssRNA (+)	RT-PCR
Human enterovirus (HEV)	ssRNA (+)	RT-PCR
Parainfluenzavirus (PIV)	ssRNA (−)	RT-PCR
Human metapneumovirus (hMPV)	ssRNA (−)	RT-PCR LAMP
Human adenovirus (HAdV)	dsDNA	PCR
Human coronavirus (NL63, HKU1, OC43, 229E)	ssRNA (+)	RT-PCR LAMP
SARS-coronavirus (SARS-CoV)	ssRNA (+)	RT-PCR
MERS-coronavirus (MERS-CoV)	ssRNA (+)	RT-PCR LAMP
WU (WUPyV) and KI (KIPyV) polyomavirus	dsDNA	PCR
Human bocavirus (HBoV)	ssDNA	PCR

(+), positive sense; (−), negative sense; ARI, acute respiratory infection; ds, double-stranded; LAMP, loop-mediated isothermal amplification; RT-PCR, reverse transcription polymerase chain reaction; ss, single-stranded.

was then replaced by the A(H1N1)pdm09 virus, an influenza A subtype that has remained mostly NI susceptible. Oseltamivir resistance is more likely to develop in subjects given oseltamivir prophylaxis or in immunocompromised patients with prolonged viral shedding.[18,19]

Oseltamivir resistance is most often associated with the histidine for tyrosine amino acid substitution at position 275 in the neuraminidase gene (His275Tyr) in influenza A/H1N1 viruses, and the glutamine to valine substitution and arginine to lysine substitution at positions 119 (Glu119Val) and 292 (Arg292Lys) respectively for influenza A/H3N2 viruses. Other substitutions that have been identified include isoleucine to arginine, lysine or valine at position 223 (Ile223Arg/Lys/Val), serine to asparaginase at position 247 (Ser247Asn) and isoleucine to valine at position 117 (Ile117Val). These substitutions, in combination with His275Tyr, confer even higher levels of resistance to NIs.[20]

Antiviral resistance can be determined using various NAAT methods including reverse transcription polymerase chain reaction (RT-PCR), rolling circle amplification and sequencing techniques.[21,22] High resolution melting (HRM) analysis and pyrosequencing are more widely used in clinical virology laboratories compared to conventional or next-generation sequencing (NGS), which were used to detect the single nucleotide polymorphisms (SNPs) outlined above. Compared to HRM analysis, pyrosequencing has the added advantage of estimating the relative proportions of susceptible wild-type and resistant mutant viruses in mixed population samples.[22]

Epidemiological and phylogenetic typing

NAATs can be used to explore the genomic relationships of existing or novel respiratory viruses. Such analyses allow greater resolution between and within species type to determine the origin and evolution of respiratory viruses, aid outbreak investigations by demonstrating transmission events, advance pathogenic understanding, guide discovery and subsequent detection of antiviral resistance and assess vaccine effectiveness.[23–27] Molecular typing methods generally involve PCR followed by nucleotide sequencing of partial or whole genomes.

Over a 5 year period, phylogenetic analysis of 156 complete genomes of influenza A/H3N2 viruses demonstrated the presence of multiple clades co-circulating in New York State. Multiple lineages from a common haemagglutinin gene ancestor were circulating following distinct reassortment events.[28] At a 180-bed Japanese hospital, investigators demonstrated nosocomial transmission of two genetically distinct influenza A/H3N2 variants by analysing haemagglutinin sequences over a 5 week period. These data were used to identify lapses in, and reaffirm the importance of stricter infection control measures.[29]

Full length analyses of neuraminidase and haemagglutinin genes of influenza A(H1N1)pdm09 viruses during the 2011 influenza season showed that they were distinct compared to viruses that were circulating during the influenza pandemic of 2009, but associated with viruses collected from Newcastle, Australia, at the time of transmission of oseltamivir resistant A(H1N1)pdm09 viruses in the community.[26]

Similar techniques have also identified the circulation patterns of influenza B viruses, an observation that assists with understanding influenza vaccine composition and effectiveness.[30]

Specimen collection and pre-analytical issues

NAATs can be performed on upper and lower respiratory tract samples including NTS, nasopharyngeal swabs, nasopharyngeal aspirates (NPA), throat gargles, bronchoalveolar lavage fluid and pleural fluid. Sputum is not a preferred specimen due to its viscosity, but a recent study showed higher mean viral loads for influenza A, respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) when sputa were processed using a 'dunk and swirl' method compared to NTS. This method involves dunking a sterile swab into sputum and swirling the swab into sterile water, which is subsequently processed.[31]

Respiratory specimen type and the age of the subject tested can affect the performance of NAATs.[32,33] The sensitivity of NAATs may be increased when lower respiratory tract or paediatric samples are tested. The detection of viruses from respiratory samples is also affected by the time between the onset of symptoms and specimen collection. Respiratory viruses are more likely to be detected when specimens are collected soon after symptom onset as viral loads are generally higher early in the illness, especially in paediatric samples. The quality of sample collection is especially important in respiratory tract infection, and training in sampling is recommended. However, patient self-collected samples such as throat washings in severe acute respiratory syndrome-coronavirus (SARS-CoV) infection may reduce transmission risks to healthcare workers.[34]

Testing stools may complement testing of respiratory samples for viruses able to replicate outside the respiratory tract. SARS-CoV RNA was detected in stool but not respiratory samples for more than 10 weeks after symptom onset, whilst avian influenza A/H5N1 and A/H7N9 RNA (but not human seasonal influenza viruses) have been detected in 50–78% of stool samples.[12,35,36] Quantitation of RSV RNA in blood collected from patients that have undergone haematopoietic stem cell transplantation (HSCT) may also predict poor outcomes and guide antiviral therapy.[37]

The volume and method of nucleic acid extraction from submitted specimens can also affect NAAT performance. Different extraction methods may be more suited to recovering RNA, DNA or total nucleic acids. More recently, commercial extraction-independent assays for the detection of influenza