

**REVIEW ARTICLE**

Diagnostic Methods of Respiratory Virus Infections and Infection Control

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호흡기 바이러스 감염의 진단법과 감염관리

박창은

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ABSTRACT

Respiratory viruses (RVs) cause infections in hospital environments through direct contact with infected visitors. In infection control, it causes major problems of acquired infections in hospitals by respiratory viruses. The surveillance data derived from clinical laboratories are often used to properly allocate medical resources to hospitals and communities for treatment, consumables, and diagnostic product purchases in the institutions and public health sectors that provide health care. An early diagnosis is essential in infection with respiratory viruses, and methods that can be used in diagnostic methods using respiratory samples include virus culture, molecular diagnosis, and analysis. A microchip provides a new strategy for developing a more diverse and powerful technology called point-of-care testing. The importance of the respiratory system should be applied strictly to the infection control guidelines to ensure the occupational health and safety of health care workers. Evidence of clinical efficacy, including this study, is challenging the long-standing paradigm for infection propagation. Additional assistance will be needed for frequent tests to detect respiratory viruses in inpatients who have begun to show new respiratory symptoms indicating infections requiring efforts to control the infection.

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INTRODUCTION

Guidance on respiratory protection was put forward under the assumption that it is presumed to be a mode of transmission and that influenza and other pathogens are transmitted in only one mode [1]. However, the paradigm of droplet or air diffusion is based on outdated experiments in the 1940s, and it was con-

cluded that only large droplet nuclei were found at close distances to the patient, and smaller droplet nuclei and airborne particles were found at greater distances [2]. Influenza A virus belongs to the Orthomyxoviridae family and has a genome with eight segments. Subtypes are categorized by the genetic and antigenic properties of the hemagglutinin (HA) and neuraminidase (NA) membrane glycoproteins: 18 HA types (H1-H18) and 11 NA (N1-N11) types are distinguished today.

Environmental sampling has been performed for influenza virus and human coronaviruses (including severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2; Coronavirus Disease-19]) in a number of

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field settings. The importance of indirect contact transmission is uncertain compared with other transmission routes, principally direct contact transmission, droplet, and airborne routes. Influenza virus, SARS-CoV and probably middle east respiratory syndrome coronavirus (MERS-CoV) are shed into the environment at concentrations far in excess of the infective dose, they can survive for extended periods on surfaces, and sampling has identified contamination of hospital surfaces.

For contaminated surfaces to play a role in transmission, a respiratory pathogen must be shed into the environment, have the capacity to survive on surfaces, transfer to hands or other equipment at a concentration above the infectious dose, and be able to initiate infection through contact with the eyes, nose or mouth [3].

Splash and contact are thought to be the main mode of transmission of seasonal influenza. Therefore, the World Health Organization (WHO) and Centers for Disease Control and Prevention (CDC) guidelines recommend medical masks during routine patient care, while N95 respirators are recommended for procedures and other high-risk situations where aerosols may be generated [4].

Nucleic acid amplification tests (NAATs) are predominantly used given their increased sensitivity, specificity, breadth, and reduced turnaround time to pathogen detection. Multiple NAAT methods are used in the diagnostic virology laboratory for the diagnosis of viral aetiologies of respiratory infections including PCR, RT-PCR, real-time RT-PCR (rRT-PCR). Loop-mediated isothermal amplification (LAMP) relies on auto-cycling strand displacement to generate DNA or RNA (using reverse transcriptase). Isothermal amplification has been used for the detection of several respiratory viruses including human and avian influenza viruses, respiratory syncytial virus (RSV), human metapneumovirus (hMPV), human coronavirus-NL63 and MERS-CoV [5-7]. Unlike rRT-PCR, LAMP assays cannot optimally detect multiple respiratory

viruses simultaneously.

Recently, the direct utility of the FilmArray detected 20 targets of viral and atypical pathogens present in respiratory samples, including 17 respiratory viruses as a POC test in our emergency department [8].

In early diagnosis, the first essential step is early, simple and reliable detection for respiratory viruses. The purpose of this study is to review recently reported papers on the clinically used method and provide information on the selection of appropriate test method for infection control guideline.

MAIN ISSUE

1. Infection control of viruses

RSV infection can present as a variety of clinical syndromes including upper respiratory tract infections (URTIs), bronchiolitis, pneumonia, exacerbations of asthma and viral-induced wheeze. MERS-CoV and Ebola virus disease (EVD) are not airborne infections, yet the CDC recommendation of using respirators to protect healthcare workers (HCWs) recognises the uncertainty around transmission [9]. The CDC initially recommended medical masks for Ebola, but changed their guidelines when US HCWs became infected, amidst unrest and challenges to the prior guidelines [10]. In contrast, the WHO recommends medical masks for MERS-CoV and Ebola WHO. Infection prevention and control during health care for probable or confirmed cases of novel coronavirus (nCoV) infection despite having older guidelines for filoviruses which recommended respirators.

Given the lack of effective antiviral therapy and vaccines, infection control measures remain the most important modality to prevent human-to-human transmission of SARS. Early isolation of suspected patients is important to prevent nosocomial transmission [11]. Transmission of influenza can occur through three routes. Water droplets (large particles that are too large for inhalation into the lungs and quickly sink to the ground or other surface within 2 m

from the source); Aerosol or droplet nuclei (small particles less than 5 μm can float in the air much longer and potentially inhale into the lower respiratory tract); And contact (transmission of infectious particles to the mucosa, either directly or indirectly through contaminated objects).

The American Academy of Pediatrics (AAP) guideline recommends decontamination of hands with alcohol-based gel before and after every patient contact when managing patients with RSV. The virus is spread predominantly by infected nasal secretions RSV spreads readily within hospitals to infect children and staff [12].

The incubation periods stated in the observation and experimental studies reviewed (Table 1) [13, 14]. the viruses detected on the laboratory respiratory viral panel, rapid influenza. There has been no established incubation period for metapneumovirus in the literature. Handwashing is simple, cheap, and highly effective in preventing nosocomial respiratory syncytial virus infections. Early diagnosis, a strict cohorting and contact isolation policy, and prospective surveillance contribute to the reduction of nosocomial RSV infection [15]. A systematic review of infection control measures to prevent nosocomial RSV transmission found that multicomponent interventions appeared to be most effective, with reductions in transmission of up to 50% [16]. The infection pattern of transmission according to various respiratory viruses (Table 2)

[17-23].

2. Comparison diagnostic methods of respiratory virus

Most of the information available about the total pathological signs of RSV infection is from observations in bronchial alveolar lavage (BAL) fluid, biopsy, and autopsy samples extracted primarily from severe cases. RSV detection and surveillance has not been standardized worldwide, RSV detection approaches have historically been inaccessible, and prevention strategies are inefficient. Airways damaged by viral infection are susceptible to secondary bacterial infection [24]. In a series of studies examining patients admitted to hospitals with RSV infections, between 17.5 and 44% of patients also tested positive for a lower respiratory tract bacterial coinfection, with *Streptococcus pneumoniae* (Gram positive) and *Haemophilus influenzae* (Gram negative) being the most common bacterial isolates [25]. Association of IL-33 with asthma during RSV infection. Bacterial coinfections during RSV infection are common, and prior colonization with potentially pathogenic bacterial species may be a risk factor for severe RSV infection [26]. Compared to RSV infection alone, a bacterial coinfection combined with RSV infection correlates with more severe disease [27]. Standardization of RSV detection methods will be required when an RSV vaccine is approved for broad dissemination. A consensus on ideal RSV detection and surveillance technologies is clearly needed, preferably before a vaccine is approved for community administration. This will likely include a breadth of factors,

Table 1. Incubation period of various virus infection by systematic review (days) [13, 14]

| Virus | Median periods | Range of incubation periods |
|------------------------------|----------------|-----------------------------|
| Adenovirus | 5.6 | 4.8~6.3 |
| Coronavirus human (non-SARS) | 3 | 2.0~5.0 |
| Coronavirus SARS-associated | 5 | 2.0~10.0 |
| Influenza A | 1.4 | 1.3~1.5 |
| Influenza B | 0.6 | 0.5~0.6 |
| Measles | 10 | 8.0~14.0 |
| Metapneumovirus | Not available | 3~6 |
| Parainfluenza | 2.6 | 2.1~3.1 |
| Respiratory syncytial virus | 4.4 | 3.9~4.9 |
| Rhino/enterovirus | 1.9 | 1.4~2.4 |

Table 2. Transmission modes of viral factors

| Pathogens | Primary source | Other source | References |
|-----------------------------|----------------|---------------------|------------|
| Adenovirus | Contact | Fecal-oral, Droplet | [17] |
| Coronavirus | Droplet | Contact | [18] |
| Parainfluenza virus | Contact | Droplet | [19] |
| Influenza A virus | Droplet | Contact, Airborne | [20] |
| Influenza B virus | Droplet | Contact | [21] |
| Respiratory syncytial virus | Contact | Droplet | [22] |
| Rhinovirus A/B | Airborne | Droplet | [23] |

Table 3. Comparison evaluation methods of diagnostic method by reported paper

| Test | Assay | Population (yr) | Comparison method | Sensitivity (%) | Specificity (%) | References |
|-----------------------------------|--|-----------------|---|-----------------|-----------------|------------|
| 2nd-generation point of care test | 3M rapid detection RSV BD Veritor system | All ages <6 | Composite standard: cell culture and DFA | 86.3 | 95.8 | [27] |
| | RSV K-SeT antigen test Sofia | <6 | Prodesse ProFlu+RT-PCR Laboratory-developed RT-PCR Traditional cell culture | 81.6 | 99.1 | [28] |
| Rapid molecular test | Sofia POC | <18 | RT-PCR | 79.1 | 95.8 | [29] |
| | Cepheid Xpert Flu/RSV | Not defined | Laboratory-developed RT-PCR | 87.7 | 94.7 | [30] |
| Multiplex molecular test | Cepheid Xpert Flu/RSV | Not defined | Laboratory-developed RT-PCR | 85 | 97 | [31] |
| | Sofia RSV FIA AdvanSure | Not defined | Laboratory-developed RT-PCR | 97.9 | 100 | [32] |
| Multiplex molecular test | Seeplex RV15 ACE | Not defined | Composite reference | 90.6 | 99.4 | [33] |
| | ResPlex II Panel v2.0 | Not defined | Composite reference | 80.8 | 98.7 | [34] |
| | Seeplex RV15 | 0~17 | Composite reference | 96.8 | 100 | [35] |
| | xTAG RVP | 0~17 | Composite reference | 94.7 | 100 | [35] |
| | xTAG RVP Fast | 0~17 | Composite reference | 84.0 | 100 | [36] |
| | xTAG RVP Fast | 0~17 | Composite reference | 100 | 97.7 | [36] |
| | xTAG RVP Fast | 0~17 | Composite reference | 88.2 | 100 | [36] |
| | Simplexa FluA/B and RSV | 0~84 | Laboratory-developed real-time RT-PCR | 91.7 | 100 | [36] |
| | | Adult patients | Laboratory-developed real-time RT-PCR | 94.7 | 99.2 | [37] |
| | | | Laboratory-developed RT-PCR | 91.3 | 98.9 | [38] |

Table 4. Comparison of different respiratory virus molecular detection methods

| Assay | Target | Character | Sensitivity (%) | Specificity (%) | References |
|---|--|---|--------------------------|--------------------------|------------|
| Influenza A and B virus multiplex RT-PCR | <i>m</i> <i>h1</i> (944 bp) <i>h3</i> (591 bp) | PCR based on testing 1033 specimens | 92 | 84 | [40] |
| | <i>ha</i> | Multiplex RT-PCR detects 19 viruses using Luminex xMAP system on testing 544 specimens | 96.4 for A 91.5 for B | 95.9 for A 96.7 for B | |
| Avian influenza virus H5N1 real time RT-PCR | <i>m</i> | M-Chip low density microarray using oligos on testing 43 isolates | 97 | 100 | [41] |
| RSV multiplex RT-PCR | <i>pol I</i> | Multiplex PCR detects 7 viruses (influenza A and B, PIV-1-3, RSV and adenovirus etc.) and IFA based on testing 598 specimens (including 123 RSV positives) | 100 | 100 | [42] |
| Rhinovirus multiplex RT-PCR | <i>5'-ncr</i> | Multiplex RT-PCR detects 19 viruses and second PCR, sequencing performance based on testing 554 specimens | 100 | 91.3 | [43] |
| QuickVue® RSV (dipstick immunoassay) | glycoprotein, fusion protein | Samples (nasopharyngeal aspirate specimens, NUCLESENS® easyMag® platform [bioMérieux®, MA, USA]) and real-time PCR performed on ABI 7300 instrumentation (AgPath-ID One-Step RT-PCR master mix kit [Ambion®, TX, USA]) Nasopharyngeal swabs (FLOQSwabs™ [Copan Flock Technologies, Brescia, Italy]) | 90.1 | 98.8 | [44] |

including but not limited to specificity, sensitivity, processing time, complexity, and cost. This is a summary of the comparative evaluation of various respiratory viruses based on the previously reported papers (Table 3) [28-39].

3. Molecular diagnosis of respiratory virus

These tests should increase our understanding of the epidemiology of respiratory virus infections. Finally, genotyping assays to detect antiviral resistance, which have also recently appeared, will provide clinicians with new information to improve patient management. Detection of RSV in these assays may be coupled to a relatively small number of targets (e.g., influenza A/B virus) or may occur in extensively multiplexed panels (e.g., eSensor respiratory viral panel [RVP] and Luminex RVP panels). While some assays such as the Verigene respiratory virus nucleic acid test allow for discrimination between RSV A and RSV B, other assays do not distinguish between these RSV types (e.g., Prodesse ProFlu).

Rapid molecular methods include integrated nucleic acid extraction, amplification, and detection systems. Although they are generally unable to handle high workflows, these tests have excellent sensitivities and specificities compared to molecular tests run in high-complexity laboratories. Recent antigen detection tests utilize specialized detection approaches to improve test characteristics. The chromatographic assay detects the RSV-F glycoprotein is intended for use on nasopharyngeal swabs. With both described second-generation POC tests, negative specimens should be verified with another method (e.g., viral culture or an FDA-approved molecular test) according to the manufacturer's instructions. This is the result of comparing and organizing the effectiveness of the molecular diagnosis method for respiratory viruses based on the recently reported paper (Table 4) [40-45].

CONCLUSION

Quantitative assays have recently been developed for viral testing of respiratory specimens. It offers the possibility to establish a correlation between the severity of the virus and the clinical disease. Influenza virus replicates in the epithelial cells of the upper and lower airways. Infected hosts release viruses from the environment when they breathe, splash, cough, and sneeze, causing virus-containing particles of 0.01 to 500 μm in size to form sprays, causing infection [45]. Respiratory virus symptoms such as sneezing and coughing result in the generation of virus-containing particles, in a size continuum from 1 to 500 μm . Whereas the generation of small droplet nuclei has traditionally been associated with 'aerosol-generating procedures' [46].

Respiratory viruses can cause relatively mild upper respiratory infections to severe lower respiratory tract infections with pneumonia and bronchitis. Since respiratory viral infections show similar symptoms and signs, it is difficult to discriminate the causative virus from clinical features alone, and because the contagious power is very high, it may cause a pandemic in a short period of time. Therefore, it is important to diagnose respiratory virus infection early to prevent unnecessary antibiotic abuse and to establish appropriate infection control measures. Automated, miniaturized new test equipment that has the advantages of existing molecular diagnostic tests has been continuously developed, and the importance of point-of-care-testing (POCT) in the medical environment has increased. The development of POCT brought many changes in the form of treatment in tertiary medical institutions, and many examinations were subjected to real-time examinations and reports [47].

The superiority of respirators should be reflected in infection control guidelines to ensure the occupational health and safety of healthcare workers (HCWs) [48]. There is a need for a more evidence-based approach to updating guidelines and ensuring consistency between

different guidelines. Clinical efficacy data are a higher level of evidence than theoretical paradigms of transmission, and show better protection afforded by respirators. Rapid influenza diagnostic test (RIDT) is an antigen-based test developed to quickly diagnose influenza virus infection in the diagnosis of field examination. Currently, FDA-approved RIDT can detect or distinguish influenza A or B virus, detect only type A influenza virus, or detect both influenza virus although it cannot distinguish between type A and type B [49].

The use of the FilmArray RP panel may be helpful in reducing antibiotic prescriptions in outpatients with respiratory tract infection (RTIs) and anti-influenza prescriptions in patients with nondetected pathogens may be reduced [8].

A limitation of molecular diagnostic tests is that point mutations in the primer binding site can lead to false negative results. The reason is that most primers are selected from conserved regions of the genome. Molecular diagnostic tests can be applied to emerging viruses after the viral genome has been sequenced.

요약

호흡기 바이러스는 감염된 방문자와의 직접적인 접촉을 통해 병원 환경에서 감염된다. 감염관리 분야에서 호흡기 바이러스에 의한 병원내 획득 감염의 주요한 문제를 유발한다. 임상 검사실에서 파생된 감시 데이터는 또한 의료 서비스를 제공하는 기관과 공중 보건 분야에서 치료, 소모품 및 진단 제품 구매를 위해 병원과 지역 사회에 의료자원을 적절하게 배분하기 위해 종종 사용된다. 호흡기바이러스의 감염에서 조기 진단은 필수적이며 호흡기 검체를 사용하는 진단법에 활용될 수 있는 방법에는 바이러스 배양, 분자 진단 및 분석 등이 포함된다. 랩온어칩(LoC)/마이크로칩은 보다 다양하고 강력한 기술인 차세대 현장검사 시험법을 개발할 수 있는 새로운 전략으로 제공한다. 호흡기계의 중요성은 의료관련 종사자의 직업적 건강과 안전을 보장하기 위해 감염 관리 지침에 엄격히 적용되어야 한다. 이 연구를 포함하여 점점 더 많은 임상적 효능 증거가 감염 전파에 대한 오랜 패러다임에 도전하고 있다. 바이러스의 감염 가능성을 의심하는 새로운 호흡기 증상이 시작된 입원 환자로부터 호흡기 바이러스를 탐지하기 위한 빈번한 검사에 대해 추가 지원이 요구되고 감염

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