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Alexander J. McAdam

Developments in Tissue Culture Detection of Respiratory Viruses **623**

Alexander J. McAdam and Ann Marie Riley

Viral culture is the historical gold standard for detection of most viruses that cause respiratory tract infections. Viral culture remains valuable because it is reasonably sensitive for most respiratory viruses, and it is cheaper and less technically demanding than nucleic acid amplified tests. The disadvantages of conventional viral culture using multiple tubes of cell lines are that it is labor intensive, moderately expensive, and slow. Advances in viral culture include the introduction of new cell lines, which can be more sensitive or convenient than previously used cell lines, and the use of shell-vial culture for respiratory viruses. Shell-vial culture is as sensitive as conventional culture for most respiratory viruses and it has a much shorter turn-around time. The shorter turn-around time increases the clinical utility of these cultures.

Developments in Immunologic Assays for Respiratory Viruses **635**

Marie Louise Landry

In most hospitals, clinics, and doctor's offices, immunologic assays are the only tests performed on site for the diagnosis of respiratory viruses. More than other methods, immunoassays have been shown to affect patient management and save costs, aiding early administration of antiviral therapy, reduction in unnecessary tests and antibiotics, and earlier discharges. This article discusses the major immunologic methods employed for respiratory virus diagnosis, recent developments in immunoassays and sample collection, and current test algorithms.

Antigen-Based Assays for the Identification of Influenza Virus and Respiratory Syncytial Virus: Why and How to Use Them in Pediatric Practice **649**

Nicola Principi and Susanna Esposito

This article describes the clinical and socioeconomic relevance of influenza (IV) and respiratory syncytial virus (RSV) in pediatrics, the characteristics and limitations of currently available assays, and the impact of rapid diagnostic tests. This article shows that rapid tests for the detection and identification of IV and RSV in the respiratory secretions of infants and children are useful in the diagnosis of common, and possibly severe diseases, such as influenza and bronchiolitis. The tests' specificity and sensitivity make them most reliable when the prevalence of influenza or RSV infection is high, which suggests that their routine use should be restricted to the

peak periods of viral circulation. The most recently marketed tests are similarly effective in identifying viruses, and so pediatricians should choose those that are less expensive, less time consuming, and easier to perform and to interpret.

Utilization of Nucleic Acid Amplification Assays for the Detection of Respiratory Viruses

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Sue C. Kehl and Swati Kumar

Viruses are major contributors to morbidity and mortality from acute respiratory infections in all age groups worldwide. Accurate identification of the etiologic agent of respiratory tract infections is important for proper patient management. Diagnosis can be problematic, because a range of potential pathogens can cause similar clinical symptoms. Nucleic acid amplification testing is emerging as the preferred method of diagnostic testing. Real-time technology and the ability to perform multiplex testing have facilitated this emergence. Commercial platforms for nucleic acid amplification testing of respiratory viruses include real-time polymerase chain reaction (PCR), nucleic acid sequence-based amplification, and loop-mediated isothermal amplification. Multiplex PCR with fluidic microarrays or DNA chips are the most recent diagnostic advance. These assays offer significant advantages in sensitivity over antigen detection methods and in most cases also over traditional culture methods. A limited number of assays, however, are commercially available, thus laboratory developed assays frequently are used. This article reviews the performance of commercially available assays and discusses issues relevant to the development of in-house assays.

Emerging Molecular Assays for Detection and Characterization of Respiratory Viruses

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Wenjuan Wu and Yi-Wei Tang

This article describes several emerging molecular assays that have potential applications in the diagnosis and monitoring of respiratory viral infections. These techniques include direct nucleic acid detection by quantum dots, loop-mediated isothermal amplification, multiplex ligation-dependent probe amplification, amplification using arbitrary primers, target-enriched multiplexing amplification, pyrosequencing, padlock probes, solid and suspension microarrays, and mass spectrometry. Several of these systems already are commercially available to provide multiplex amplification and high-throughput detection and identification of a panel of respiratory viral pathogens. Further validation and implementation of such emerging molecular assays in routine clinical virology services will enhance the rapid diagnosis of respiratory viral infections and improve patient care.

The Human Bocaviruses: A Review and Discussion of Their Role in Infection

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Brian D.W. Chow and Frank P. Esper

Respiratory tract infections are a leading cause of morbidity and mortality worldwide. The human bocavirus (HBoV) is a newly recognized human

parvovirus first reported in 2005. Since its discovery, this virus has been associated with upper and lower respiratory tract disease and gastroenteritis worldwide. This article is a comprehensive review of what is known about HBoV. It includes an evaluation of diagnostic modalities, symptoms occurring in affected patients, and a discussion as to whether HBoV is responsible for identified clinical manifestations. The article reviews the incidence and effect of coinfection and updates on related members (HBoV-2 and HBoV-3) recently reported. Understanding of respiratory viruses such as HBoV remains vitally important to the health of adult and pediatric patients.

Recently Discovered Human Coronaviruses

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Brigitte A. Wevers and Lia van der Hoek

In marked contrast to their historical classification as relatively harmless, common cold-causing, respiratory pathogens, human coronaviruses (HCoVs) are associated with more severe clinical complications, as emphasized by the discovery of severe acute respiratory syndrome-associated CoV (SARS-CoV) in 2003. Still, their precise pathogenic potential is largely unknown, particularly regarding the most recently identified strains HCoV-NL63 and HCoV-HKU1, and definite proof for their etiology remains a major challenge. This article focuses on the characteristics of the five HCoVs that are known, and summarizes current knowledge of their pathogenic potential in people, with an emphasis on the interactions between these viruses and their cognate receptors on susceptible target cells.

Respiratory Syncytial Virus Vaccine Development

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Yoshihiko Murata

Respiratory syncytial virus (RSV) is a clinically significant cause of respiratory tract disease, especially among high-risk infants and immunocompromised and elderly adults. Despite the burden of disease, there is no licensed prophylactic RSV vaccine. The initial efforts to develop an RSV vaccine involved formalin-inactivated virus preparations that unexpectedly caused vaccine-enhanced disease in clinical trials in RSV-naive children. Over the last 40 years, cautious and deliberate progress has been made toward RSV vaccine development using various experimental approaches, including live attenuated strains and vector-based and viral protein subunit/DNA-based candidates. The scientific rationale, preclinical testing, and clinical development of each of these approaches are reviewed.

Respiratory Viruses in Bronchiolitis and Their Link to Recurrent Wheezing and Asthma

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Jonathan M. Mansbach and Carlos A. Camargo, Jr

Bronchiolitis is the leading cause of hospitalization for children younger than 1 year of age and these hospitalized children have an increased risk for developing childhood asthma. It remains unclear, however, which

children who have severe bronchiolitis (eg, an episode requiring hospitalization) will develop recurrent wheezing or asthma. Although many environmental and genetic factors may play a role in the pathway from bronchiolitis to asthma, this article focuses on the viruses that have been linked to bronchiolitis and how these viruses may predict or contribute to future wheezing and asthma. The article also discusses vitamin D as an emerging risk factor for respiratory infections and wheezing.