

**LABORATORY METHODS FOR THE DIAGNOSIS OF UPPER AND LOWER  
RESPIRATORY TRACT INFECTIONS IN CHILDREN**

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**Abstract:** Sharp breath of the roads infections whole world along children's illness and death main from the reasons one to be global on a scale respirator infections from 0 to 5 years old was 2 million children close to death reason to be guess Approximately 80% of these respiratory infection cases are caused by viral pathogens such as influenza A and B, respiratory syncytial virus A and B, parainfluenza virus types 1-3, adenovirus, rhinovirus, human metapneumovirus, and others .

**Keywords:** Electron microscopy, breath airways , red blood cells, influenza, respiratory virus, adenovirus, rhinovirus, human metapneumovirus .

**Research objective:** Laboratory diagnosis of respiratory tract infections in children modern methods of identification Analytical review of modern scientific literature sources covering the issues.

**Materials and styles.** An analysis of 19 literature sources was conducted on this topic.

**Introduction:** Electron microscopy is one of the oldest direct detection techniques used in developed countries for both clinical virus diagnosis and the study of viral ultrastructure and pathogenesis . Historically, EM has played an important role in the identification of novel virus strains in several epidemic situations, such as the coronavirus associated with the severe acute respiratory syndrome (SARS) outbreak. However, despite several advantages, the use of EM is limited in respiratory virus diagnostics because it is expensive, laborious, time-consuming , and requires more time (approximately 3–16 h including sample preparation) and is often insensitive compared to other diagnostic methods. In addition, EM requires tight control of experimental conditions, high concentrations of viral particles ( $> 10^5 \text{ L}^{-1}$ ), and considerable technical skill and experience for accurate analysis.

**Culture method** Virus detection by cytopathic effect and hemosorption in cell culture has been the “gold standard” for the diagnosis of respiratory viral pathogens for decades. Viruses such as adenovirus, influenza A/B, RSV, and human parainfluenza viruses are the most common respiratory viruses isolated and identified by cell culture . The traditional tube culture method is useful for growing a wide variety of viruses, including new or unknown viruses, but it takes days and often weeks to produce results. Over the years, modified cell culture methods, such as the improved shell flask method of centrifugation, have reduced the cycle time from 5 to 10 days to 24 hours . Shell flask culture using combined cell lines

allows the simultaneous detection of multiple respiratory viruses and has similar sensitivity to conventional culture for parainfluenza 1-3 (87% vs. 83%) and influenza A/B (78% vs. 75%). %), and significantly higher sensitivity for RSV (73% vs. 42%). Despite these advantages, many clinically important viruses are difficult to grow in culture (e.g., rhinovirus and coronavirus) and can give variable results. In addition, multiple freezing and thawing of samples prior to testing can reduce viral titers, which can affect culture growth. Therefore, compared with molecular tests, traditional tube and shell vial culture methods are laborious, exhibit high false negative rates, and have longer turnaround times, making viral culture less clinically relevant.

Culture is also the gold standard for the detection of atypical (bacterial) respiratory pathogens, followed by identification and antimicrobial susceptibility testing using a variety of manual or automated methods. Bacterial culture can also often be insensitive, especially when the adequacy of the specimen is not determined by Gram stain or when specimens are collected after antibiotic exposure. Bacterial culture is also labor-intensive, requires considerable technical expertise, and if antibiotic susceptibility testing is performed, the turnaround time is typically 48–96 hours and may therefore be considered inadequate for optimal patient care and management of effective antimicrobial therapy.

**Methodology:** Express immunoenzyme test (EIT) Express immunoenzyme test (EIT) can deliver test results in less than 30 minutes and is typically performed in a POC setting, thus allowing the test results to be incorporated into a clinical decision-making algorithm. Four major EIT formats (latex agglutination, horizontal flow devices, lateral flow devices) Among the immunochromatographic methods (and optical immunoenzymes), lateral flow immunoenzyme (LOIF) is the most versatile and popular. RIAs are relatively inexpensive, easy to perform, and most of them have been waived in the United States under the Clinical Laboratory Improvement Act (CLIA) guidelines, making them This makes them invaluable in outpatient settings, primary care, emergency settings, and low-resource settings. Currently, commercially available RIAs are limited to the detection of influenza A virus, influenza B virus, and RSV. Many studies have shown that RIAs overall poor susceptibility to influenza and RSV (44-95%); however, they have a high average specificity (90 to 95%) compared to cell culture.

In the pediatric population, commercially available immunoassays have demonstrated high sensitivity (93%) for the detection of RSV, and a systematic review of published studies has shown that RSV RIA sensitivity in children is relatively higher than in adults (81%).). The higher sensitivity may be due to the fact that pediatric patients often shed higher titers of respiratory viruses and for longer periods of time than adults. Despite the low overall sensitivity, RIAs are considered a valuable diagnostic tool in the emergency department because they can significantly reduce the length of stay, additional ancillary tests, and antibiotic prescription for children who test positive for influenza.

**Direct Fluorescent Antigen Tests** Direct fluorescent antibody (DFA) testing of nasopharyngeal wash specimens is a rapid and reliable method for the detection of respiratory viral infections. Commercial DFA kits have shown high sensitivity and specificity for several respiratory viruses, including hMPV (95 and 100%), adenovirus (62 and 100%), RSV (94 and 96%), and parainfluenza viruses (88 and 99.7%). The results can be subjective and require technical expertise for accurate interpretation (16). The high

specificity of DFA (99–100%) suggests that the test can be used as a reliable detection method, especially for RSV in children during the early days of illness, as shown by Shafik et al .

**Serological tests** Pathogen-specific antibodies usually appear approximately 2 weeks after the initial infection and can be detected by serological tests. Serological tests can successfully detect antibodies to most respiratory pathogens, such as RSV, adenovirus, influenza A and B, parainfluenza viruses 1–3, and others, and can detect mixed infections in children with hospitalized acute respiratory infections, with the exception of infants, in whom antibody responses are usually not detectable. However, serological tests have been reported to be significantly less sensitive for the detection of parainfluenza virus and adenovirus compared with molecular methods such as RT-PCR ( 18 ). RT-PCR has identified 40% more samples from pediatric patients who were positive for at least one respiratory virus than those detected by fluorescent antibody assay (FA). FA testing, in addition to RT-PCR, has been useful for epidemiological studies because it increases the likelihood of detecting acute viral infections and has been used to accurately assess respiratory viruses other than influenza in children.

Serological testing for bacteria is particularly challenging, especially for the detection of atypical bacterial agents such as *Mycoplasma pneumoniae* . Serological tests have limited clinical utility, with varying sensitivities (14%–77%) and specificities (49%–97%) compared with PCR. The clinical utility of serological tests is further limited because they are not available for acute and convalescent infections to monitor seroconversion or detect a fourfold increase in antibody titer . requires sera . In addition, serological tests are often not useful for detecting recurrent viral infections because serum IgM levels are low due to vaccines or repeated exposure to circulating viruses. For optimal virus-specific IgM testing, an acute-phase serum sample should be obtained early in the course of the illness.

**Low-Plex Integrated Test Systems** Low-plex integrated respiratory test systems typically target 1–4 pathogens per assay. Comparative studies evaluating the performance of these assays have reported >90% concordance between different commercial platforms and in many cases >95% sensitivity and specificity. The total processing time for each sample for these assays ranges from ~1–2 hours and The number of samples that can be tested in a standard 8-hour shift depends on the number of instruments available in the testing laboratory. Smaller panels targeting multiple pathogens that can be run independently (random access) or simultaneously (random batch) may be a viable option to control laboratory costs. Therefore, when considering different test options, it is advisable to consider other assay characteristics, such as ease of use, panel composition, total run time, practical time, and cost, in addition to clinical performance, before implementing the assay in different clinical settings.

**Discussion:** Nowadays, there are a large number of rapid and cost-effective genotyping kits with high throughput, which can enable large-scale genotyping of rare blood groups. In addition, some new detection methods can also detect rare blood groups using a small amount of samples, such as Kleenex paper towel-based elution and direct flow methods, bromocresol green paper strip, nitrocellulose-based lateral flow technique (MD multicardR), microflow strip-based analytical device, SPR technique, etc. These approaches to detect rare blood groups may shed light on the most promising strategies.

**Conclusion:** Molecular tests have significantly improved the diagnosis of respiratory pathogens and are being accepted as the new “gold standard.” Although these tests have become very popular, factors such as patient population (adults, children, and immunocompromised), laboratory size, purpose of the test (routine or emergency care), and cost/benefit ratio must be considered before performing a specific analysis. For example, during the 2012–2013 influenza epidemic, two cases were identified at Memorial Sloan Kettering Cancer Center on January 1, 2013, which resulted in hospitalization. Despite appropriate infection control measures, five additional cases were identified a few days later, on January 7, 2013.

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