Detection of respiratory viruses

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Acute respiratory diseases account for an estimated 75% of all acute morbidities in developing countries, and most of these are caused by viruses. Upper respiratory tract infections (URTI) are among the most common infections in children, occurring 3–8 times per year in children under 5 years of age, and often causing acute asthma exacerbations or acute middle ear infections. The U.S. Centers for Disease Control and Prevention's National Vital Statistics Report indicates that there are 12–32 million episodes of URTI each year in children under 2 years of age. Viral respiratory tract infections can be caused by 18 different types of viruses. These include conventional agents such as influenza A and B, respiratory syncytial virus (RSV) types A and B, parainfluenza virus (types 1–4), adenovirus (50 types), the "common cold" viruses (including 2 coronaviruses and over 100 types of rhinovirus), and 6 viruses discovered since 2001—metapneumovirus, 3 coronaviruses [including severe acute respiratory syndrome (SARS)– associated virus], bocavirus, and avian influenza virus (H5N1).

Clinical disease

All of the 18 virus groups indicated above can cause a full range of respiratory tract infections, from the typical mild common cold with signs and symptoms of a runny nose and sneezing to more severe presentations such as pharyngitis, laryngitis, bronchitis, or pneumonia. The severity of disease varies depending on the level of immunity of the individual, and is generally more severe in immunocompromised patients [for example, those with human immunodeficiency virus (HIV); transplant recipients receiving antirejection drugs; diabetics; and the elderly]. As the immune competency of individuals and their ability to fight infection and cancer begins to decline in the fourth decade of life, the elderly patient is particularly susceptible to virus infections, which are often fatal in these circumstances.

Traditional diagnostic methods

In clinical practice, a specific virus is often not identified due to the lack of a laboratory test that is sensitive enough to detect it. As mentioned above, there have been six new viruses discovered since 2001. These have historically accounted for a significant proportion of infections where no virus could be detected in the absence of tests. The rhinoviruses and two coronaviruses discovered in the mid-1960s were "orphaned" by the medical community until recently since they were not considered to cause life-threatening illnesses. Typically, viruses were identified by their shape and size and classified into families; for example, the coronavirus has a "crown" around its surface made up from a single protein (**Fig. 1**). Virology laboratories have historically diagnosed only six conventional respiratory viruses using traditional methods. These methods include, first and foremost, virus

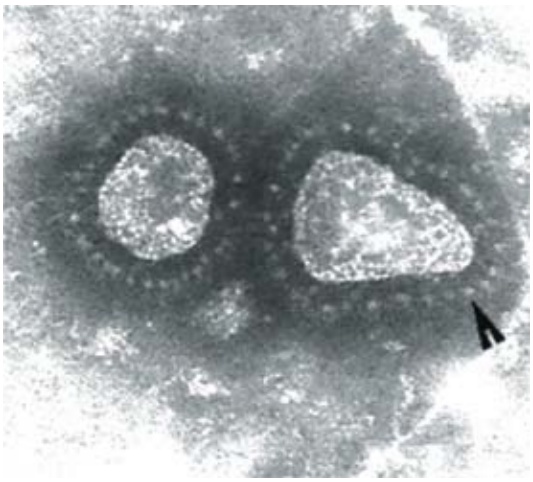

Fig. 1 **Electron microscopic image of SARS coronavirus magnified 140,000 times. The arrow indicates the presence of the "corona" or crown formed by a single surface protein.**

Advantages and disadvantages of various methods for detecting respiratory viruses

Methods*	Advantages	Disadvantages
Cell culture	Low sensitivity	Not all viruses are culturable
DFA	Gold standard method	Positivity rates vary by laboratory
ELISA	Provides point of care result	Insensitive
NAAT	Highly sensitive	Expensive
M-PCR	Detects several viruses	Expensive

*Abbreviations: DFA = direct fluorescent antibody; ELISA = enzyme-linked immunosorbent assay; NAAT = nucleic acid amplification test; M-PCR = multiplex polymerase chain reaction.

isolation in cell culture using up to four different cell lines (not all viruses grow in all cell lines). Preformed cell cultures in 15-cm-long (6-in.) tubes are inoculated with specimens, placed in a roller drum where they are constantly rotated for 10 days, and viewed daily under a microscope for virus-induced cell damage, indicating the presence of a virus. In theory, culture can be sensitive enough for detection of a single living virus particle. However, at the same time, sensitivity can be lost when specific antibodies in the specimen neutralize the virus, preventing its growth (see table).

The second most important detection method has been direct fluorescent antibody (DFA) staining for the presence of virus-infected cells. This method involves collecting epithelial cells from a nasopharyngeal swab, fixing them to a glass microscope slide, staining with individual antibodies labeled with a fluorescent tag, and viewing the slide with a fluorescent microscope (**Fig. 2**). This method has sensitivities ranging from 65 to 90% for the six viruses commonly detected. Sensitivity can be compromised if the specimen is collected too late in the course of infection when the number of cells containing viral proteins is diminished. The third most commonly

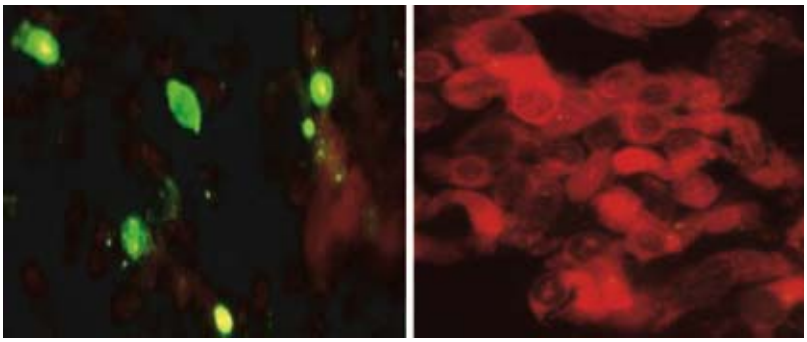

Fig. 2 **Direct fluorescent antibody (DFA) staining of an influenza-positive specimen (*left*) showing the presence of virus-infected cells (green) stained with a fluorescent monoclonal antibody, and a negative specimen (*right*) showing uninfected cells stained with a red counterstain.**

used method is shell vial culture. This involves inoculating an aliquot of the specimen onto a preformed cell monolayer in a small vial containing a mixture of two susceptible cells, which is then centrifuged to enhance virus attachment and entry. The centrifugation-assisted inoculation of the cells increases the amount of viral proteins produced, allowing staining to be performed at 24–48 h and thus providing a test result to be obtained significantly earlier than the 7–10 days necessary for traditional cell culture. Rapid enzyme-linked immunosorbent assays (ELISAs), in which a monoclonal antibody conjugated to an enzyme is used to rapidly detect and quantify the presence of an antigen in a sample, have been developed as bedside tests for influenza and RSV, but these are too insensitive for routine use.

Molecular methods

The traditional methods mentioned above have been the cornerstone for diagnosis used by virology laboratories around the world for the past 25 years. The introduction of nucleic acid amplification tests (NAATs) for respiratory viruses starting in the late 1980s has heralded a new era in diagnosing respiratory virus infections. The first NAAT for respiratory viruses was developed for influenza, and used a nucleic acid amplification method called polymerase chain reaction (PCR), developed in 1983 by Kary B. Mullins, who was later awarded the Nobel Prize in Chemistry in 1993. Within a decade, NAATs were developed for all of the respiratory viruses, and most used PCR; however, other amplification schemes such as nucleic acid–sequence-based amplification (NASBA), strand displacement amplification (SDA), transcription-mediated amplification (TMA), and loop-mediated isothermal amplification (LAMP) have also been used. For all NAATs, the total nucleic acid is first extracted from the respiratory tract specimen using a variety of methods and the viral ribonucleic acid (RNA) is copied into a complementary deoxyribonucleic acid (cDNA) using an enzyme called reverse transcriptase. The cDNA is then amplified by PCR using virus-specific oligonucleotide primers, resulting in a billion copies of DNA that can be easily detected by a variety of common laboratory methods. Following the emergence of five new human respiratory viruses since 2001, there was a need for new diagnostic tests to detect these viral pathogens and NAAT filled this need. Early comparisons of molecular and traditional methods clearly indicated that the

molecular methods were more sensitive than the traditional methods, often diagnosing up to 30% additional infected patients. Molecular testing methods also provided test results for clinicians often within 1 day (as compared with 2–5 days needed for traditional methods), thus improving their management of patients.

The next major advance in diagnostics was the development of multiplex PCR (M-PCR) for the detection of several different viruses in a single test. M-PCR uses multiple oligonucleotide primers, with one pair for each virus to be detected. Since M-PCR will detect several different viruses, a method is required to identify which virus is present in the specimen. This is done using a microarray (a collection of several different DNA oligonucleotides) that is either spotted onto microscope slides or cartridges (gene chips) or immobilized onto microspheres (microfluidic arrays) that are each uniquely labeled using a mixture of fluorescent dyes and identified by lasers. Each element in the array (a spot or microsphere) consists of a unique oligonucleotide (representing a unique virus) that will bind individual PCR products for each virus type or subtype. A positive specimen will generate an amplification product that is hybridized to one of the elements of the microarray and detected by a laser. One M-PCR called the xTAG™ RVP (respiratory viral panel) has recently received clearance as an in vitro diagnostic device by the U.S. Food and Drug Administration. This test was designed to identify 20 different respiratory virus types and subtypes and uses over 30 primers for target amplification and identification. Although M-PCR tests are slightly more expensive than single NAATs, they have the advantage of being able to detect many different viruses in a single test, as well as being able to detect dual infections occurring in about 10% of patients and even triple infections that are not often seen with traditional methods. NAATs using M-PCR and microarray technology offer unprecedented power for the laboratory, and are well on the way to becoming the pillars of diagnostic virology for the present century.

See also: CLINICAL MICROBIOLOGY; COMMON COLD; INFLUENZA; NUCLEIC ACID; OLIGONUCLEOTIDE; PARAINFLUENZA VIRUS; RESPIRATORY SYNCYTIAL VIRUS; RESPIRATORY SYSTEM; RESPIRATORY SYSTEM DISORDERS; RHINOVIRUS; VIRUS; VIRUS CLASSIFICATION.

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Keywords

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