



CHAPTER 5

VIRUSES AS THE CAUSE OF UPPER AND LOWER ARI IN CHILDREN: GENERAL CHARACTERISTICS AND DIAGNOSIS

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I. INTRODUCTION

Acute respiratory infections (ARI) of the upper respiratory tract are one of the leading causes of child mortality throughout the world, but especially in the developing countries, causing approximately 15 million deaths per year (1).

Among the numerous etiologic agents described, viruses are recognized as the predominant etiologic agents in ARI (2), in adults as well as children and in both developing and industrialized countries (3).

Although bacteria have been posited as the predominant etiologic agent of ARI in the developing countries (4), an international multicenter study coordinated by the Board on Science and Technology for International Development of the National Academy of Sciences of the United States determined that viral etiology is present in a larger proportion of cases than bacterial etiology (5), the percentages of viral identification ranging between 17% and 44% of ARIs in under-5 children, depending on the country. The most frequently isolated viruses were the respiratory syncytial virus (RSV), which accounted for between 11% and 37% of total cases studied; adenoviruses, for between 1% and 7%; parainfluenza viruses types 1 and 3, for between 1% and 3%; and influenza viruses types A and B, for between 1.4% and 4.3% (6-9).

It has been demonstrated that the same clinical picture may be caused by different agents, and the same agent is capable of causing a wide range of syndromes. The most common viral causes of upper ARIs are the rhinoviruses and the coronaviruses. In lower ARIs, the most com-

mon etiologic agents are influenza viruses, parainfluenza viruses, RSV, and adenoviruses (Table 1). However, viruses other than those mentioned may also cause upper and lower respiratory infections in children. These include *Herpes simplex*, Epstein-Barr virus (EBV), measles virus, and mumps virus. The viral infection itself may cause mild or severe illness, or it may be complicated, predisposing the patient to subsequent bacterial infections (10). Viral pneumonia is more common than bacterial pneumonia, but it carries a much lower risk of death (11).

II. DIAGNOSIS OF VIRAL ARI

Viral respiratory infections have traditionally been diagnosed by detecting the etiologic agent during the illness or by determining the antibody titer during convalescence. This diagnostic method is complex owing to the large variety of agents that may cause ARI, although it has been greatly simplified by the existence of techniques for the direct detection of virus in nasopharyngeal aspirates (12).

Isolation of the virus in cell cultures coupled with identification by immunochemical techniques is considered the method of choice or the standard method for viral diagnosis. Nevertheless, this method is costly and relatively slow (sometimes taking more than a week). It has been possible to shorten the time required to obtain viral culture results through low-speed centrifugation of cell cultures inoculated with the specimen, followed by identification by immunofluorescence.

Technology for the diagnosis of viral respiratory infections has advanced rapidly, even more so than for bacterial infections, which has led to the development of new viral diagnostic techniques that are sufficiently rapid, sensitive, and specific. In addition, as a result of the development of antiviral chemotherapy agents, such as amantadine for influenza virus, ribavirin for RSV, and others which are still being tested, rapid and accurate etiologic diagnosis is becoming increasingly necessary for patient management.

The past several years have seen the development of direct diagnostic methods that make it possible to detect the presence of virus in clinical specimens within a few hours. These procedures are immunofluorescence (IF), both direct and indirect; enzyme-linked immunosorbent assay (ELISA) of similar sensitivity; time-resolved fluoroimmunoassay (TR-FIA); polymerase chain reaction (PCR); and nucleic acid hybridization. These methods may yield a diagnosis within 4-24 hours after the specimen is obtained.

Serological methods of detecting viral antibodies are not the preferred method for diagnosing respiratory infections because they are not as sensitive and because the humoral immune response to these viruses, which do not produce viremia, is generally rather weak. In addition, the need to use paired specimens of serum (i.e., specimens from the period of acute illness and from the period of convalescence) means that the results do not influence the therapeutic management of the patient. In any case, serological diagnosis is useful for epidemiological studies, vaccine tests, and clinical tests of new antiviral drugs, in which cases it is important to detect

both clinical and subclinical infections. Generally speaking, the ELISA technique for detecting IgG antibodies in paired serum specimens is the most sensitive method for diagnosing ARI of viral origin.

a) Clinical specimens for diagnosis

To obtain an accurate virological diagnosis, proper selection, extraction, shipment, storage, and processing of the specimen are essential. Because the period during which the virus is excreted tends to be brief, it is important to take specimens during the first few days of the illness. A specimen taken too late may yield a false negative result. Moreover, owing to the relative frequency with which these viral infections are contracted in hospitals, specimens from hospitalized patients should be obtained at the time the patient is admitted to avoid uncertainty as to whether the virus found is attributable to the disease that prompted the hospitalization or to a nosocomial infection.

The nasopharyngeal aspirate (NPA) is the specimen of choice for identifying the viruses that cause ARI because it provides an appropriate number of infected cells. NPAs are obtained by inserting a sterile catheter into the patient's nostrils and using a vacuum pump or a syringe to aspirate nasopharyngeal secretions, which are placed in a sterile conical flask. The specimen, hermetically sealed and packed in ice, is sent to the virology laboratory for processing. It should not be frozen.

Other specimens that can be used are bronchoalveolar, nasal, or pharyngeal washings. Swab specimens generally contain fewer cells and are therefore not the best choice for diagnosis. Nevertheless, both nasal and throat swabs can be used together to increase the yield.

b) Specimen processing

The objective of good specimen processing is to optimize the isolation and identification of any viruses that may be present in the specimen (Figure 1). The first step is a careful separation of the mucus. To detect viral antigens through ELISA or viral nucleic acids through polymerase chain reaction (PCR) or molecular hybridization, use of uncentrifuged NPA is recommended. To detect virus by indirect immunofluorescence (IF) or isolation in cell cultures, the specimen is centrifuged at 4° C for 10-15 minutes at 3,000 g (3,000 times gravity). The supernatant is used to isolate the virus in cell cultures. The cells in the pellet are washed, deposited on a slide, and fixed with cold acetone for 10 minutes, and processed for IF.

The various methods for identifying the viruses that cause upper and lower ARIs in children are described below.

b.1) Isolation in cell cultures

It is recommended that this method be used whenever it is available in the laboratory because it is the method of choice when several viruses are possible etiologic agents. The specimens should be kept at between 4° and 6° C but not frozen. The resid-

ual material should be stored fractionated at 70° C or less in case the original specimen is needed again.

As can be seen in Figure 1, the technique consists of inoculating an aliquot¹ of 0.2 ml of the NPA supernatant in a monolayer into the various cell cultures, from which the growth medium has been previously removed. After inoculation, maintenance medium is added (without bovine serum and with the addition of trypsin in the case of cells sensitive to the growth of influenza and parainfluenza virus) and the cultures are incubated at 34°-35° C. They should be checked daily for 11 days to detect the appearance of cytopathic action (CA) on the monolayer. Four to seven days after the infection, a hemadsorption (HAD) test is performed to detect influenza and parainfluenza virus, using red blood cells from guinea pigs on specific cultures (MDCK, LLC-MK2, and others). Those cultures on which cytopathic action and/or hemadsorption are observed are processed for virus identification by IF. The primary cell cultures or established lines that permit isolation of the various respiratory viruses are numerous and will be mentioned below in the description of each virus.

An increase in viral infectivity and a reduction of the time needed to obtain results has been achieved through low-speed centrifugation of the cell cultures inoculated with the specimen from the patient, which increases the sensitivity of the culture. After a short incubation to allow reproduction, fluorescent staining is done to identify the viral agent by IF. The combination of the two techniques has been shown to have the same specificity and sensitivity as traditional isolation in tube cell cultures, but the time required to obtain results is reduced from 10 days to 2 or 3 (13-17).

b.2) Immunofluorescence (IF)

Immunofluorescence, both direct and indirect, is a simple technique that allows for rapid identification of numerous viruses. In direct IF, the specific antiviral serum is labeled with fluorescein. In indirect IF, a specific serum is made to react against the antigen of the virus to be detected (produced in animals) and then an antibody targeted against the immunoglobulin of the animal species used in the first step, labeled with fluorescein, is added.

WHO has coordinated multicenter studies for the development and use of monoclonal antibodies in the diagnosis of viral ARIs by IF. Tests conducted with IF diagnostic kits in 16 different laboratories demonstrated their effectiveness (18).

Specific polyclonal or monoclonal antisera are now commercially available for the identification of most respiratory viruses (19). Mixtures of monoclonal antibodies have been shown to have high sensitivity and specificity, comparable to any reference method, for the identification of viral antigens in clinical specimens. A minimal loss of

1 A part or proportion of something.

sensitivity can be expected when they are compared to high-quality polyclonal antibodies, but monoclonal antibodies permit easier and much more accurate reading (18).

b.3) Enzyme-linked immunosorbent assay (ELISA)

Several enzyme-linked methods for the identification of respiratory viruses have been developed in recent years, with various results. These tests are used to detect antigens in clinical specimens. Using the sandwich principle, specimens are introduced into tubes or onto special plastic slides on which the “capture” antibody specific to the antigen has been adsorbed. Then another antibody specific to the antigen, but labeled with an enzyme (peroxidase and alkaline phosphatase are most commonly used), is added. When the substrate is added, enzymatic activity is detected by a change of color that can be read visually or with an ELISA reader. Monoclonal antibodies have improved the sensitivity and specificity of these methods and have helped to extend the use of ELISA as a diagnostic method (20-23). This method can also be used to detect antibodies in serum.

b.4) Hybridization with probes

A more recent diagnostic approach is analysis of viral genomes through hybridization with specific nucleic acid probes to detect the virus. The labeled probe is applied to the clinical specimen and, if a complementary chain of viral nucleic acid exists, hybridization occurs and is detected according to the labeling system used (radioactive or biotinylated probes). These probes may be prepared by different methods, depending mainly on the virus to be investigated. The recent trend has been toward use of recombinant nucleic acid clones or synthetic oligonucleotides that represent specific sequences of the viral genome under study (3, 24, 25).

b.5) Polymerase chain reaction (PCR)

This method makes it possible to detect very small amounts of virus through the amplification of sequences of viral genomic deoxyribonucleic acid (DNA) present in the specimen. The process requires the use of complementary oligonucleotides of conserved genomic sequences of the virus called primers, and a heat-stable DNA polymerase enzyme. The reaction produces millions of copies from an single sequence of viral DNA, which can then be detected with the naked eye (by means of staining with ethidium bromide) or through hybridization (radioactive or enzymatic). The use of PCR is still experimental, especially for influenza viruses, RSV, and enteroviruses (3).

b.6) Time-resolved immunofluorescence assay (TR-IFA)

This recently developed method for the detection of respiratory viruses is, to date, the most sensitive solid-phase assay. It has made it possible to increase the sensitivity of fluorescence by eliminating nonspecific background fluorescence and producing a

fluorescence of greater intensity and decay time through the use of europium chelate. Its simplicity and rapidity derive from the fact that the specimen is incubated simultaneously, for only an hour, with the capture antibody and the specific antibody labeled with europium chelate. However, the high cost of the equipment required to perform TR-FIA has limited its use to reference laboratories (3).

III. RESPIRATORY SYNCYTIAL VIRUS (RSV)

RSV is a paramyxovirus of the genus *Pneumovirus*. The viral particle is pleomorphic and enveloped, with a diameter ranging from 150 to 300 nm (26). The nucleic acid of RSV is a single strand of RNA with negative polarity. It has no hemagglutinating, hemadsorbent, hemolytic, or neuraminidase action. The virus is very sensitive to changes of temperature, which should be borne in mind when attempting to isolate it in cell cultures.

To date, one serotype and at least two antigenic strains or subgroups (A or 1 and B or 2) of RSV have been described. The main difference between the subgroups is found in the glycoprotein G. The two circulate simultaneously in the population, and the clinical and epidemiological importance of these antigenic strains is not clear (27-29). It is likely that the antigenic diversity of the two subgroups of RSV has some influence on the susceptibility of children to sequential infection these strains. In some countries, the existence of epidemic patterns in which the A and B subgroups alternate in two-year cycles has recently been demonstrated (3).

The incubation period for respiratory disease is 4-5 days. The virus reproduces in the nasopharynx and can persist in the infected child for up to three weeks. The virus may spread from the upper airway to the respiratory tract through the respiratory epithelium or through aspiration of infected secretions. To date, RSV has not been found to produce viremia (26). An intact immune system appears to be necessary to overcome the infection, although the infection in children may occur even in the presence of maternal antibodies. Reinfections are common at all ages and sometimes occur only a few weeks after the initial infection (30).

Evidence of RSV infection has been found in all geographic areas studied. Annual epidemics occur, mostly during the cold months. During epidemics there is a rise in cases of bronchiolitis and pneumonia and an increase in hospitalization of young children for lower respiratory infections. Before entering school (by 4 or 5 years of age), most children have been infected with RSV. Age, sex, and socioeconomic conditions influence the severity of the illness but not the attack rate.

Bronchiolitis is the illness most frequently caused by this virus, especially in children between 6 weeks and 6 months of age. This disease is uncommon during the first 6 weeks of life (31). The primary RSV infection may be manifested as a lower respiratory infection, pneumonia, bronchiolitis, tracheobronchitis, or upper respiratory infection, often accompanied by fever or otitis media. The infection is rarely asymptomatic. Pneumonia and bronchiolitis may be difficult to differentiate, and many infants may appear to have both syndromes (32). RSV causes epidemics that affect a very large proportion of children, some of which will require

hospitalization. These children shed high titers of virus for several days, which frequently leads to nosocomial infections, especially in infant wards. Manifestations of the infection may range from mild febrile illness of the upper respiratory tract to severe illness of the lower respiratory tract and even death (32). In adults infected with RSV, the disease may occur in the upper or lower respiratory tract. In the elderly, RSV may cause bronchopneumonia.

RSV grows in a wide variety of human and animal cells. The cell lines in which RSV can be isolated are Hep-2, HeLa, Vero, LLC-MK2, MRC-5, BSC-1, and CV-1, as well as in primary cultures of bovine kidney or human embryonic kidney. The virus induces the formation of characteristic syncytia in Hep-2 cells (23, 26).

Direct and indirect immunofluorescence with poly- or monoclonal antibodies has proved to be quite useful for the detection of virus antigens in desquamated nasopharyngeal cells (17, 18, 33).

The ELISA method permits identification of positive specimens with about the same efficiency as immunofluorescence. This test can detect between 20 and 30 ng of viral protein (20). Commercial kits are currently available, although, when tested, they yielded different sensitivity and specificity values (21, 23). Tests are being conducted using polymerase chain reaction and nucleic acid probes to detect RSV in clinical specimens, as well as the detection of viral nucleoprotein through the application of monoclonal antibodies with the TR-FIA technique (3).

IV. ADENOVIRUSES

The family *Adenoviridae* encompasses a large number of species of human and animal origin which are widely distributed in nature. According to the current classification, the numerous members of the family are grouped in two genera: *Mastadenovirus* and *Aviadenovirus*. The genus *Mastadenovirus* includes the human adenoviruses and many others isolated from various animals. All of them are characterized by specificity to their host species and by tremendous genetic variability. They generally infect their hosts through the conjunctiva or the digestive mucosa. To date, 47 different species or serotypes of human adenovirus have been recognized (34).

After their discovery by Rowe in 1953 (35), different criteria were used to classify these agents, until finally they were grouped in six subgenera (A-F). All adenoviruses share the same general structure: symmetrical icosahedral viral particles, unenveloped, with a average diameter of 80 nm. The viral genome is double-stranded DNA. The replication of DNA and the transcription and maturation of the adenoviruses take place in the cell nucleus, within which there is an accumulation of structural proteins in "inclusion bodies," characteristic of alveolar cells during adenovirus pneumonia (36, 37).

Adenoviruses are found throughout the world. The different serotypes cause illness in different age groups, and the severity varies according to geographic area. The infections are generally self-limiting, and the clinical characteristics of the illness depend on both the host and the serotype involved. Approximately 65% of adenovirus isolations are from children under the age of 4 (36, 38).

Adenoviruses are responsible for 5% of cases of respiratory infection in children under 4 and 10% of respiratory infections requiring hospitalization in the same age group (37). They may cause pharyngitis, conjunctivitis, laryngotracheobronchitis, and bronchiolitis, but acute pneumonia is undoubtedly the most serious clinical manifestation, especially among small children, in whom it may be fatal (36). The serotypes most frequently associated with these illnesses are Ad3, Ad4, Ad7, and Ad21, as well as Ad2 and Ad5 (37). Sequelae of significant residual pulmonary damage, including bronchiectasis and obliterating bronchiolitis, have been described (39).

Infection with certain serotypes may also cause pertussis-type clinical syndrome that is indistinguishable from the disease of bacterial etiology except through the detection of the viral or bacterial agent (40).

These serotypes represent an increasingly serious problem among immunocompromised hosts. Five new serotypes (42-47) associated with AIDS cases have recently been described (34).

Adenoviruses are stable viruses which can be recovered from clinical samples with relative ease. Nasopharyngeal aspirate, nasal swabs, and material from biopsy or necropsy are suitable specimens for their isolation. Recovery of the virus can be considered a sign of recent infection, and it is frequently associated with symptomatic illness.

Primary cultures, diploid cell lines, and continuous human cell lines (A-549, Hep-2, HeLa, Kb cells) can be used to diagnose an adenovirus infection. A characteristic cytopathic effect is produced with rounding and clustering of the cells and formation of intranuclear inclusion bodies (37). The isolate can be identified through immunofluorescence.

The detection of viral genomes through hybridization with specific nucleic acid probes is a technique that is currently being used for the diagnosis of adenoviruses (24, 25).

A four-fold or greater rise in serum antibody titers between the acute and convalescent phases of the illness, detected through complement fixation or ELISA, is strongly indicative of the presence of adenovirus infection. ELISA is currently considered the best test for detection of immune response in children (37).

V. PARAINFLUENZA VIRUS

The parainfluenza viruses are classified in the genus *Paramyxovirus* of the family *Paramyxoviridae*. They are viruses that contain RNA in a nucleocapsid with an envelope derived from the membrane of the host cell. They are pleomorphic, measuring 100-200 nm in diameter, with spikes that have hemagglutinin and neuraminidase (HN protein) functions, activities which are inseparable. They also have spikes that are responsible for hemolytic and fusion (F protein) action, making them capable of fusing cells and hemolyzing certain types of erythrocytes (41), which differentiates them from influenza viruses.

Four types have been identified: 1, 2, 3, and 4. These viruses are recognized by their cytopathic action on infected cells or through hemadsorption action with red blood cells from guinea pigs on monolayers of infected cells.

These viruses are frequent causes of respiratory infections of varying severity, which depends on the type of virus and, especially, on the age of the host, in direct relation to the production of primary infections or reinfections. Types 1, 2, and 3 are recognized as the principal causal agents of croup, although they may also cause pneumonia and bronchiolitis (42).

The parainfluenza viruses, especially types 1 and 2, are responsible for more than 50% of cases of croup and obstructive laryngitis. Bronchiolitis, associated with type 3, may occur after the first month of life; cases occur year round, but there are also epidemic outbreaks. Parainfluenza viruses are found worldwide. The illness they cause is generally mild. All four types may cause respiratory infections, and reinfection, particularly with serotype 3, has been demonstrated in adults and children. These agents generally infect the very young. Types 1 and 2 cause epidemics in the fall. Type 3 infections occur year round. A serologic study found that 60% of children have been infected with type 3 parainfluenza by age 2 and approximately 80% have been infected by age 4, the majority asymptotically (43). The high rate of infection suggests that the virus spreads rapidly. Like RSV, parainfluenza may cause severe illness in infants, but the severity of illness diminishes after age 3 (44). Illness so severe as to require hospitalization is rare (42).

The period of virus shedding is generally short, and these viruses must therefore be isolated from tracheal or nasopharyngeal aspirates taken early in the illness. The clinical specimen is inoculated on different cell lines, preferably primary monkey kidney lines (although established lines of LLC-MK2 or MDCK may also be used), maintaining the cells in a serum-free medium. A cytopathic effect will sometimes occur, but at other times it will be necessary to detect the presence of virus in cultures through hemadsorption tests (HAd), using red blood cells from guinea pigs at 4° C (45). The virus can be identified through IF, hemagglutination inhibition, or neutralization with specific antisera.

The IF technique has been used successfully for direct detection of viral antigen in nasopharyngeal cells (33, 46). Cross-reactions between the different types of this virus and the mumps virus are frequent and complicate serologic diagnosis, both by hemagglutination inhibition and complement fixation.

VI. INFLUENZA VIRUS

The influenza viruses belong to the family *Orthomyxoviridae* and the genus *Influenzavirus*. Specific antigenic determinants make it possible to differentiate three types: A, B, and C, all of which may cause acute respiratory illness.

The virion may be filamentous or spherical and measures 80-120 nm, with a double-layer lipid envelope derived from the host cell; projecting from the envelope are spikes, which are the glycoproteins hemagglutinin (HA) and neuraminidase (NA).

The nucleocapsid is a symmetrical helix composed of eight fragments of single-stranded RNA associated with the nucleoprotein and three polymerases. The segmented structure of the nucleic acid explains the genetic lability and the ease with which genomic rearrangement

occurs in these viruses. Influenza viruses A and B are widely distributed and occur seasonally in temperate climates. Type A virus was isolated in 1933 and type B in 1940, although there is evidence of epidemics caused by this agent 200 years ago (47).

Influenza virus infection occurs in explosive epidemics, with rapid spread of the virus in a geographic region. The main cause of the occurrence of influenza epidemics is the continual appearance of new antigenic strains derived from earlier strains, which escape previously acquired immunity and cause illness in persons of all ages. These antigenic variations are more frequent in influenza A. For several years influenza epidemics are caused mainly by one type of virus, but it is common for more than one type to circulate, whether simultaneously or sequentially.

The influenza virus shows two distinguishable patterns of distribution. One occurs when there is a major antigenic change (antigenic shift) and antigenic subtypes that are new or have not circulated in many years appear, causing influenza A pandemics at irregular and unpredictable intervals. In 1918 an estimated 20 million people died worldwide due to infection with a type A virus, which is believed to have been subtype H1N1. The most well-documented pandemics occurred in 1918, 1957, 1968, and 1977.

The other distribution pattern is due to a minor antigenic variation or trend (antigenic drift), with relatively minor and frequent changes (annually or every few years) in a subtype of influenza. The evolution of influenza viruses is neither simple nor predictable, as was believed initially; at present, it is considered that a "new" strain is not necessarily the cause of a pandemic. It has been demonstrated, for example, that the A/H1N1 strain that appeared in 1977 was genetically identical to a strain circulating in 1950s (3).

Influenza viruses A and B may cause upper and lower respiratory diseases such as tracheobronchitis and pneumonia. Many times the infection is subclinical or the symptoms are mild. The virus spreads from person to person and has an incubation period of 1-4 days. Infection with influenza C is associated with subclinical infections or moderate common colds. The C type does not cause epidemics, and fatalities are rare (47).

Diagnosis of the influenza virus can be made by inoculating nasopharyngeal secretions on primary cultures of kidney cells from various species or lung cells from chicken embryos, or in embryonated eggs. It has been demonstrated that the use of proteolytic enzymes, generally trypsin, can increase replication in continuous cell lines such as MDCK (48). Forty-eight hours after the cells are inoculated, the presence of virus can be detected through hemadsorption with guinea pig red blood cells (45). If this test is negative, the procedure should be repeated twice a week. If the result is positive or if cytopathic action is observed, the virus is identified through IF with specific antibodies. Hemagglutination can also be done on the supernatant.

IF has been used for many years to detect antigens in epithelial cells and is currently the most common method (33, 49). The time-resolved immunofluorescence assay technique has also been used for successfully for several years (50).

The PCR technique has been used to identify influenza virus in clinical specimens (51), as well as to differentiate the various types of the virus (52).

Serologic diagnosis by complement fixation or hemagglutination inhibition is useful when paired sera exist since the acute infection is accompanied by a significant rise in serum antibody titers.

VII. RHINOVIRUS

Rhinoviruses belong to the family *Picornaviridae*. They are characterized by their susceptibility to acids. To date, 100 serotypes are known (53). They measure between 20 and 27 nm and contain four structural proteins which form an unenveloped symmetric icosahedral capsid. Synthesis and maturation take place in the cytoplasm.

These viruses are the principal known cause of the common cold. They infect not only humans but also the higher primates, and they will grow in cell cultures derived from these species. Their optimal temperature for replication is between 33° and 35° C, the temperature found in the nose and upper respiratory tract.

Rhinoviruses occur worldwide and tend to be epidemic in fall and spring. The various antigenic types circulate at random. Prevailing serotypes are gradually replaced by different serotypes.

Infections begin in early childhood and continue throughout life. Infection rates vary from one or two infections per person per year among children under age 1 to 0.7 infection in adults. The virus is transmitted through contact with secretions, either hand-to-hand or through inhalation of airborne droplets (54). The illness they cause is generally mild and lasts 7 days, on average.

Diagnosis of rhinovirus is not commonly sought, but when it is necessary diagnosis is made basically through isolation in human embryonic pulmonary cells (WI-38, MRC5) or HeLa cells. The cytopathic effect is characteristic and appears at between 2 and 6 days. Precise identification is made by demonstrating acid-lability (at a pH of 2.9) and through neutralization with specific antibodies (this is done in very few laboratories and is rarely necessary). Detection of the viral genome through nucleic acid hybridization techniques and PCR has begun to be done with these viruses, in which antigen detection is not possible due to the large number of serotypes (53, 3).

VIII. CORONAVIRUSES

The coronaviruses belong to the family *Coronaviridae*, genus *Coronavirus*. The virus particle is pleomorphic and measures 80-150 nm, with petal-shaped surface projections that give it the appearance of a crown. Coronaviruses are RNA viruses and all develop exclusively in the cytoplasm of infected cells.

Coronaviruses cause colds in children and adults. The various strains have been found to cause similar illnesses. The incubation period is longer and the duration of the illness is shorter than with rhinoviruses, but the symptoms are similar. Coronaviruses rarely cause more serious lower respiratory infection.

These viruses are found worldwide and are more frequent in winter and spring. They may account for as much as 35% of all viral ARIs in peak seasons. Reinfection is common. Infection may occur at any age, although it is more frequent in children.

Currently, the virus may be diagnosed through antigen detection methods such as IF or ELISA (56). Recovery of the virus from clinical specimens is difficult. Coronavirus must be cultured on animal tissues. Some strains have been adapted to grow on human diploid fibroblastic cell cultures (56).

The most commonly used diagnostic method at present is detection of a fourfold or higher increase in the antibody titer in paired specimens, especially by complement fixation (57).

IX. ECHOVIRUS AND COXSACKIEVIRUS

Both viruses belong to the genus *Enterovirus*, family *Picornaviridae*. They share many morphological and physicochemical characteristics with the rhinoviruses. Both have an unenveloped icosahedral capsid measuring 20-30 nm, with an unsegmented RNA genome. They do differ in number of serotypes: there are 34 serotypes of echoviruses, while there are 24 types of group A coxsackievirus and 6 of group B coxsackievirus.

They can all cause febrile illness with respiratory symptoms, although over 90% of the infections caused by this group are asymptomatic. Once a host has been infected, the viruses remain in the respiratory tract for one or two weeks.

Both genera, with their large variety of serotypes, pose problems for laboratory diagnosis. The fact that they grow very slowly in cell cultures makes their identification difficult.

At present there are no readily available methods for detecting the antigens of all these viruses, and serology is generally difficult and costly (58). As a result, diagnosis of these agents of acute respiratory infection is not routinely made.

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XI. APPENDICES

Table 1. Viruses as causes of acute respiratory infections		
Syndrome	Viral etiologic agent	
	Most frequent	Less frequent
Upper respiratory infection (common cold)	Rhinovirus Coronavirus Adenovirus Parainfluenza 3	Influenza A or B Parainfluenza 1 or 2 RSV Enterovirus
Pharyngitis	Adenovirus Enterovirus	Influenza A RSV Parainfluenza 1 or 2 Rhinovirus Coronavirus
Croup	Parainfluenza 1, 2, and 3	Influenza A RSV Measles Coronavirus
Bronchiolitis	RSV Parainfluenza 3	Adenovirus Parainfluenza 1 and 2 Influenza A or B Rhinovirus
Pneumonia	RSV Parainfluenza 3 Adenovirus Influenza A	Parainfluenza 1 and 2 Rhinovirus

Figure 1. Direct etiologic diagnosis of viral ARIs



