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ORIGINAL ARTICLE

Viral Diseases of the Respiratory System and Diagnostic Methods for Particularly in Impoverished Populations of Tropical Regions

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Abstract

Respiratory contagions (RVs) beget infections in sanitarium surroundings through direct contact with infected cells. In infection control, it causes major problems of acquired infections in hospitals by respiratory system. The surveillance data deduced from clinical laboratories are frequently used to duly allocate medical coffers to hospitals and communities for treatment, consumables, and individual product purchases in the institutions and public health sectors that give health care. An early opinion is essential in infection with respiratory contagions, and styles that can be used in individual styles using respiratory samples include contagion culture, molecular opinion, and analysis. A microchip provides a new strategy for developing a more different and important technology called point- of- care testing. The significance of the respiratory system should be applied rigorously to the infection control guidelines to insure the occupational health and safety of health care workers. substantiation of clinical efficacy, including this study, is challenging the long- standing paradigm for infection propagation. fresh backing will be demanded for frequent tests to descry respiratory contagions in convalescents who have begun to show new respiratory symptoms indicating infections taking sweats to control the infection.

Keywords: Respiratory System, Viral Diseases, Disease in humans, Influenza

Introduction

Respiratory viruses are the common causative agents of disease in humans, significantly impacting mortality and morbidity worldwide, especially in children [1-4]. Around one-fifth of all childhood deaths worldwide are related to acute respiratory

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infections (ARIs), particularly in impoverished populations of tropical regions. Acute respiratory infections case-to-fatality ratios can be remarkably more significant than in temperate sites of the world. Eight human respiratory viruses commonly spread in all age groups and are identified as adapted to efficient person-to-person transmission (Table 1) [4,5]. Besides these, avian influenza virus H5N1 and SARS coronavirus (SARS-CoV) has recently emerged as public health threats. As the currently known respiratory viruses still do not account for all clinically relevant human viral respiratory illnesses, systematic searches for new agents using molecular tools are expected to discover previously unidentified agents. Although respiratory viruses cause a significant disease burden, only a few preventive or therapeutic interventions are currently available. However, recent advances in the molecular and cell biology of respiratory viruses will hopefully result in practical interventions [6].

Table 1: Common Respiratory Viruses, their Classification, Principal Syndromes, and Main Detection Methods

Virus	Classification[a]	Principal syndromes	Virus detection methods
HRSV	Groups A and B	URI, bronchiolitis, croup, bronchitis, pneumonia	Culture, Ag detection, RT–PCR
HPIV	Types 1, 2, 3, 4	URI, croup, bronchiolitis, bronchitis, pneumonia	Culture, Ag detection, RT–PCR
HRV	Species A, B, and C With 100 serotypes	URI; asthma and COPD exacerbation	Culture, RT–PCR
ADV	51 serotypes	URI, PCF, bronchitis, pneumonia	Culture, Ag detection, PCR
HCoV	Types OC43, 229E, NL(NH), HKU1	URI, bronchitis, pneumonia	Culture, RT–PCR
SARS-CoV	1 type	SARS	Culture, RT–PCR
HMPV	Groups A and B	URI, bronchitis, pneumonia	Culture, RT–PCR
HBoV	2 lineages	URI, bronchiolitis, asthma exacerbation,bronchitis, pneumonia	PCR

ADV, adenovirus; Ag, antigen; HBoV, human bocavirus; HCoV, human coronavirus; HMPV, human metapneumovirus; HPIV, human parainfluenza virus; HRSV, human respiratory syncytial virus; HRV, human rhinovirus; PCF, pharyngoconjunctival fever; SARS, severe acute respiratory syndrome; SARS-CoV, Coronavirus associated with SARS; URI, upper respiratory infection; RT–PCR, reverse transcription–polymerase chain r eaction, which includes both conventional and real-time methods.

[a]Classification in species, subgroups, serotypes, or lineages.

Influenza Virus

Influenza virus is a negative-sense single-stranded RNA virus belonging to the family Orthomyxoviridae. Influenza is a febrile disease characterized by fever, cough, upper respiratory symptoms, sore throat, rhinorrhea, nasal congestion, and systemic symptoms, including headaches, myalgia, and malaise. As many respiratory viruses can show similar signs and symptoms, it is impossible to clinically differentiate one infection from another infection. In the past two decades, several diagnostic methods have been used for diagnosing influenza virus infection. Serological tests such as the HAI test helps to detect seroconversion of influenza virus and influenza A virus-infections of either H1 or H3 [6]. Moreover, it is no longer used to diagnose influenza virus infection. Nasopharyngeal swabs and nasopharyngeal airways are the favored specimens for influenza virus detection. Historically, isolation of influenza virus was performed in embryonated hen eggs or tube cultures of Madin-Darby canine kidney (MDCK), primary monkey kidney, or A549 cells [7-10]. CPE consistent with the influenza virus can be visualized by light microscopy but is variable depending on cell types. The disadvantage of tube cultures is time-consuming to obtain a positive result, generally 4-5 days but in some cases as long as 10-14 days. Neither tube culture nor shell vial culture can provide a more rapid result in 18 to 24 h and can detect the inactivated virus. The advantage of shell vial culture is that most viruses can be recognized in 18 to 48 h compared to 4 to 14 days for traditional tube culture [8]. The disadvantage of shell vial culture is that some viruses do not replicate robustly in R-Mix cells. If viruses want to be recovered for further analysis, they need to be passaged in primary monkey kidney cells or R-Mix Too cells.

The direct fluorescent antibody is used to detect PIV types 1, 2, and 3; influenza A and B viruses; adenovirus; RSV; and, more recently, hMPV. Direct fluorescent antibody assays take 2 to 3 h to perform, but some laboratories batch specimens do not process them as they arrive, which delays the turnaround time for reporting results. Molecular tests for influenza virus observation include reverse transcriptase-polymerase chain reaction (RT-PCR) [11], nucleic acid sequence-based amplification (NASBA) [9], and loop-mediated isothermal amplification (LAMP) [11,12]. In RT-PCR, the nucleic acid is reverse transcribed into cDNA using virus-specific random hexamers or oligonucleotide primers. Random hexamers have the benefit of generating cDNA for multiple viruses available in a specimen. Also, it helps in virus discovery, as cDNA is more stable during storage than extracted RNA or intact clinical specimens. Various gene targets have been used for amplification, including the matrix, hemagglutinin, and neuraminidase protein genes. Real-time RT-PCR [11] assays for influenza virus infection offer results more quickly than endpoint assays, but many cases have reactivities equal to or better than culture. NASBA and LAMP assays for detecting influenza virus using the same targets for amplification have been developed.

Parainfluenza Virus (PIV)

PIV is a negative-sense single-stranded RNA virus. It belongs to the family Paramyxoviridae. There are four serotypes of the parainfluenza Virus that infect humans. Their pathogenesis and mode of spread are similar to that of the influenza viruses, but the genetic makeup does not undergo antigenic drift or shift. PIV1 is the primary cause of acute croup in infants and young children and causes mild upper respiratory tract infections, pharyngitis, and tracheobronchitis in all age groups. PIV2 is generally related to lower infection rates than PIV1 or PIV3 [11-16]. It has been associated with mild upper respiratory tract infections, croup in children, and, occasionally, LRTI. Infections occur predominantly in fall months. Also, PIV causes lower respiratory tract infections in the elderly and immunocompromised patients, including bone marrow recipients. Parainfluenza virus infections have historically been diagnosed by virus isolation by detecting viral antigen or RNA by direct fluorescent antibody and nucleic acid amplification test, respectively, or by serological tests such as HAI tests. Most commonly used cell lines support the growth of parainfluenza viruses, including primary monkey kidney, Buffalo green monkey kidney (BGMK), LLC-MK2, A549, and MRC-5 cells [17]. Shell vial culture using centrifugation-assisted inoculation of preformed monolayers of R-Mix cells and pre-CPE staining for viral antigens are primarily used in clinical laboratories. Viral antigens are routinely recognized in

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nasopharyngeal epithelial cells in many laboratories by direct fluorescent antibodies using a panel of monoclonal antibodies. Monoclonal antibody pools that use two fluorescent dyes can be used to identify PIV 1, 2, and 3 [18]. SimulFluor reagents have excellent specificity and sensitivity compared with individual antibodies. Most laboratories do not detect PIV4 because specific antibodies have not been approved for antigen detection. A variety of nucleic acid amplification tests for detecting parainfluenza virus have been described. Most have shown increased sensitivity compared to assays that were evaluated with only a few positive specimens. More assessments of this assay will be required to know its actual performance characteristics. Recently, four commercial multiplex assays for the detection of influenza virus and other respiratory viruses have been introduced; these include [19-21]:

- The ResPlex II assay (Qiagen)
- The MultiCode-PLx RVP assay (EraGen Biosciences)
- The Seeplex RV assay (Seegene Inc., Seoul South Korea)
- The NGEN RVA ASR kit (Nanogen Inc., San Diego, CA)
- The xTAG RVP assay (Luminex Molecular Diagnostics, Toronto, Ontario, Canada)

Human Respiratory Syncytial Virus (HRSV)

HRSV is the known human pathogen of the genus Pneumovirus, family Paramyxoviridae, first isolated in 1956 from a chimpanzee with coryza. The virus particle is surrounded by a lipid bilayer containing the glycoproteins G (receptor binding), F (membrane fusion), and SH (unknown function). The virions also contain an RNA-dependent RNA polymerase (L), a phosphoprotein (P), and a 22K protein (M2-1) (Figure 1) [20-25].

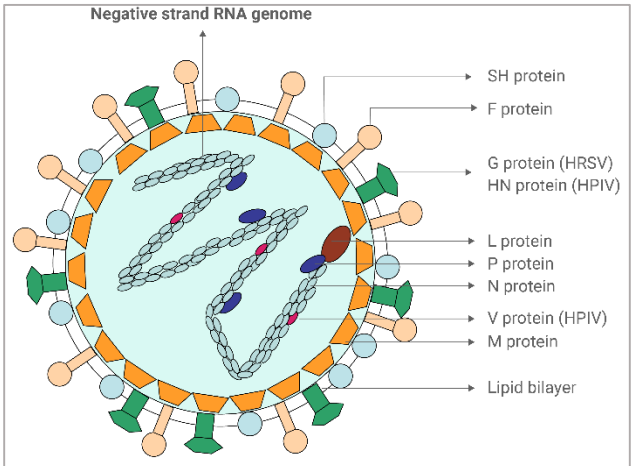

Figure 1: Schematic structure of a paramyxovirus. The virus envelope contains the F, SH, and the G/HN proteins. Underlying the envelope is the M (matrix). The nucleocapsid consists of a negative-strand RNA encapsidated by the N (nucleocapsid) protein. Associated with the nucleocapsid are L (large-polymerase), P (phosphoprotein), and V (cysteine-rich) proteins.

In addition, three nonstructural proteins (NS1, NS2, and M2- 2) are produced in infected cells. Two HRSV groups, A and B, were distinguished initially based on the antigenic differences in the attachment glycoprotein G. Later, this separation was confirmed in other genes, and phylogenetic analysis of G gene sequences allowed further division of groups into HRSV genotypes. While new genotypes continue to emerge, others that circulated previously tend to disappear. HRSV binds to cell surface glycosaminoglycans through the viral glycoprotein G, although G protein independent binding also exists. Upon virus–receptor interaction, a fusion of virus and cell membranes occurs through the binding of viral protein F to cell GTPase RhoA [23,26]. HRSV internalization is considered to be pH-independent and may happen either in plasma or in endosomal membranes. Expression of the viral protein F on the plasma membrane of infected cells promotes fusion between adjacent cells, resulting in the formation of syncytia, the hallmark of paramyxovirus cytopathic effect. Once in the cytoplasm, the negative-strand viral RNA is transcribed by the coordinated action of the proteins L, N, P, and M2-1, resulting in a set of mRNAs, which then direct the synthesis of viral proteins. The transcription of mRNAs occurs in a gradient with decreasing amounts of transcripts from the 39 ends to the 59 ends of the genome template (Figure 2) [27].

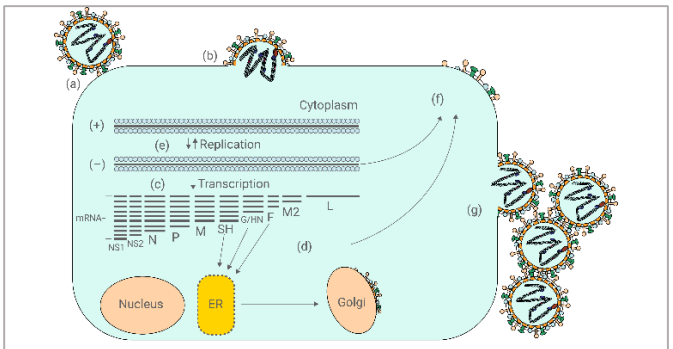

Figure 2: Schematic representation of a paramyxovirus replication cycle. (a) The G/HN protein binds to cell receptors and (b) promotes viral adsorption that results in the fusion of viral envelope with cell membrane mediated by F protein. Then genome is released into the cytoplasm. (c) A set of mRNAs is transcribed using the genome as template. (d) These mRNAs are then translated in specific proteins, some of which are glycosylated in association with the endoplasmic reticulum and the Golgi apparatus. (e) Envelope glycoproteins direct the points where new virions are formed. (f) New genomes produced by RNA replication are incorporated in newly formed virus particles. (g) The mature virus particles are released by budding from the plasma membrane.

The polymerase complex switches from mRNA transcription to viral-RNA replication at some point, generating a full-length RNA of polarity opposite to that of the genome (antigenome). Antigenome serves as a template for the polymerase complex to generate negative-sense RNA genomes of the progeny. The viral protein M2-2 seems to alter the polymerase complex in a way that may direct preference of replication over transcription. The viral matrix (M) protein mediates the association of nucleocapsids with lipid rafts containing HRSV envelope proteins in the plasma membrane, giving rise to virus budding. Viral proteins NS1 and NS2 are suppressors of type 1 interferon induction.

Diagnosis

Nasopharyngeal aspirates, nasal washings, swabs, and lower respiratory tract samples can be used as specimens for human respiratory syncytial virus detection. Sample storage and freeze-thawing significantly reduce human respiratory syncytial virus recovery in culture. The human respiratory syncytial virus can be isolated in several human cell lines, such as HeLa, HEp-2, and A549, and syncytia formation in HEp-2 cells in 3–5 days [28].

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Rapid HRSV antigen detection methods, including immunofluorescence of sloughed respiratory cells, EIA, and membrane-based chromatography, are sensitive and specific. Commercially available point-of-care sample tests require no equipment and are ideal for emergency room and primary care HRSV diagnosis. Detection of HRSV RNA by RT–PCR, both conventional and based on real-time techniques, has become widely accepted as the most sensitive diagnostic assay. In addition to sensitive qualitative results, real-time methods apply to quantitation of viral load and simultaneously detect the subtype RSV directly in clinical specimens. Other amplification methods not based on PCR, such as NASBA, have become commercially available for HRSV diagnosis. HRSV serology has limited value for case management but may be helpful in epidemiologic surveys.

Human Parainfluenza Viruses (HPIVs)

Human parainfluenza viruses are frequent causes of left respiratory tract infections in infants and children worldwide. HPI's share structural and biological characteristics with HRSV and are distributed in two genera of the family Paramyxoviridae. HPI's are classified antigenically into four types. In contrast, the HPIV-4 has subtypes A, and B. HPIV 1 and 3 types are classified in the genus Respirovirus, while HPIV 2 and 4 types are in the genus Rubulavirus. The virions are pleomorphic, single-stranded negative-sense RNA, ranging from 150-200 nm in diameter (Figure 1). The glycoprotein hemagglutinin-neuraminidase (HN), with hemagglutinin (HE) and neuraminidase activities, activates virus–cell attachment by binding to sialic acid available on the cell surface proteins. Upon separation by proteolytic enzymes, viral protein F mediates the binding of viral and cell membranes, with consequent release of the nucleocapsid in the cytoplasm. The virus life cycle happens similar to other Paramyxoviridae (Figure 2) [29]. Nonstructural proteins equivalent to NS1, NS2, and M2 of the human respiratory syncytial virus are not present in the human parainfluenza virus. HPIV zinc-binding protein V is seen in high intracellular levels but little quantity in viral particles and plays roles in controlling viral-RNA synthesis and counteracting host cell interferon type 1 response.

Diagnosis

The human parainfluenza virus is present in respiratory secretions up to 8 days from the onset of symptoms. They can be isolated from nasopharyngeal swabs, bronchoalveolar lavage, or washes/aspirates in cultures of monkey kidney primary cells or several continuous cell lines like MDCK, LLC-MK2, HEp-2, Vero, BHK, and HeLa. The presence of the human parainfluenza virus in monolayers can be confirmed by hemadsorption with guinea pig erythrocytes and immunofluorescence. Isolation of the human parainfluenza virus in the shell vial assay format has produced mixed results. If for human parainfluenza virus on exfoliated respiratory epithelial cells has been used for decades, but its sensitivity is moderate to low. Sensitive assays for detecting human parainfluenza virus RNA by RT–PCR, including multiplex format and real-time PCR-based tests, have become widely accepted. Serologic assays for human parainfluenza virus IgM and IgG are particular but are not helpful for acute patient management.

Human Metapneumovirus (HMPV)

Human metapneumovirus is now recognized as a frequent cause of community-acquired ARI in children and adults worldwide. The study of previously unidentified viral isolates that induced CPE in cultures of LLC-MK2 cells. Electron microscopy of cell cultures showing CPE revealed paramyxovirus-like particles. The sequencing of randomly primed PCR products revealed genome sequence and organization consistent with a paramyxovirus of the subfamily Pneumovirinae, related to avian pneumovirus of the genus Metapneumovirus [28-30]. HMPV particles are enveloped, pleomorphic, spherical, or filamentous particles of about 209 nm in diameter. Like other paramyxoviruses, HMPV has a negative-sense, single-stranded RNA genome, and the viral replication occurs in a gradient manner. Complete genome sequencing revealed that, in contrast to pneumoviruses, HMPV has different positioning of the genes between M and L and lacks NS1 and NS2 genes. Similar to HRSV, HMPV genes G and SH are not essential for virus replication. The protein M2-2 appears to control the switch from transcription to replication, and removal of the M2-2 gene leads to the accumulation of viral mRNAs and attenuation of viral replication.

Diagnosis

Human metapneumovirus can be isolated from nasopharyngeal washes/aspirates in LLC-MK2 cells with a medium containing trypsin. The virus grows poorly, cytopathic effect characteristically negative on hemadsorption testing, generally develops late after injection (up to 23 days). Currently, RT–PCR is the most widely used method for HMPV detection. Real-time PCR assay for HMPV has become the gold standard for being more sensitive than conventional RT–PCR [11]. HMPV-specific antibodies are now commercialized for HMPV immunofluorescence assays, but this method is not as sensitive as real-time RT–PCR; it is readily applicable in diagnostic laboratories, where IF for respiratory viruses is routinely done.

Rhinovirus

Human rhinoviruses (HRVs) are the most frequent respiratory pathogens of humans and the most commonly detected viruses in samples from typical cold sufferers. HRVs are small, non-enveloped, positive-stranded RNA viruses in the family Picornaviridae, genus Rhinovirus, distributed in two species, A (75 serotypes) and B (25 serotypes). Recently described new strains containing deletions in the VP1 region may constitute a new species C. HRV atomic structure was solved and revealed an icosahedral particle composed of 12 pentamers with a diameter of about 30 nm. Surrounding a fivefold vertex of VP1 capsid proteins from adjacent pentamers, there is a 1.2–3.0 nm wide canyon that contains the receptor-binding site for the HRVs of the significant receptor group. The HRV genome is a 7.4 kb monocistronic, single-stranded, uncapped RNA, with a small

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genomic protein (VPg) covalently linked to the 59 end and a poly (A) tail at the 39 end. The first 600–700 nt at the 59 end constitutes a highly structured untranslated region (UTR), where an internal ribosomal entry site (IRES) permits translation initiation. Following receptor binding, the viral positive-strand RNA is released into the cytoplasm. Translation of the single ORF produces one polyprotein that is cleaved co-translationally to generate three precursors – P1 that originates capsid proteins, P2 and P3, originates nonstructural proteins, and VPg [31]. Further cleavages of these precursors generate 11 end-products, and the last cleavage of VP0 into VP2 and VP4 occurs only at the final stages of virus maturation (Figure 3).

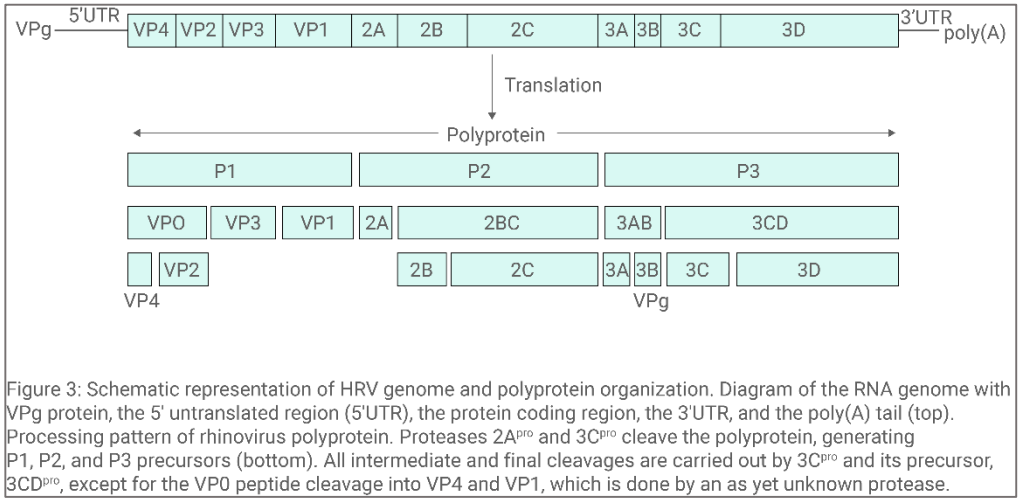

Figure 3: Schematic representation of HRV genome and polyprotein organization. Diagram of the RNA genome with VPg protein, the 5' untranslated region (5'UTR), the protein coding region, the 3'UTR, and the poly(A) tail (top). Processing pattern of rhinovirus polyprotein. Proteases $2A^{pro}$ and $3C^{pro}$ cleave the polyprotein, generating P1, P2, and P3 precursors (bottom). All intermediate and final cleavages are carried out by $3C^{pro}$ and its precursor, $3CD^{pro}$, except for the VP0 peptide cleavage into VP4 and VP1, which is done by an as yet unknown protease.

One of the P3 cleavage products, 3D, is an RNA-based RNA polymerase that will produce a negative-stranded full-length copy of the genome to be used as a template for producing an expanding pool of positive-stranded RNAs. The positive-stranded RNAs can be either translated into viral proteins or packaged as a genome into newly assembled virions [32]. The HRV replication cycle occurs in association with vesicle membranes in the cytoplasm, and mature virions are released upon the cell in lysis (Figure 4).

Until recently, the recognized 100 HRV serotypes were classified according to receptor specificity into three groups: The primary group with 90 serotypes, whose receptor is intercellular adhesion molecule-1 (ICAM-1); the minor group with ten serotypes, whose receptor is the low-density lipoprotein receptor (LDLR); and HRV-87 that shares properties with human enterovirus 68 and utilizes sialic acid residues on cell proteins as a receptor. The range of HRV serotypes and species by the currently accepted classification system based on sequencing and phylogenetic comparisons rather than on receptor usage or antiviral susceptibility is expected to expand as more sequence information is obtained from field strains. HRV is stable for days on environmental surfaces and is resistant to ethanol, ether, chloroform, and nonionic detergents, but is sensitive to UV light, pH lower than 5, and halogens like chlorine, bromine, iodine, and phenolic disinfectants.

Diagnosis

Human rhinovirus can be detected in respiratory secretions by isolation in cultures of susceptible cell lines. Cell lines of primate origin support human rhinovirus propagation, but certain strains of HeLa cells and human embryonic fibroblasts provide higher

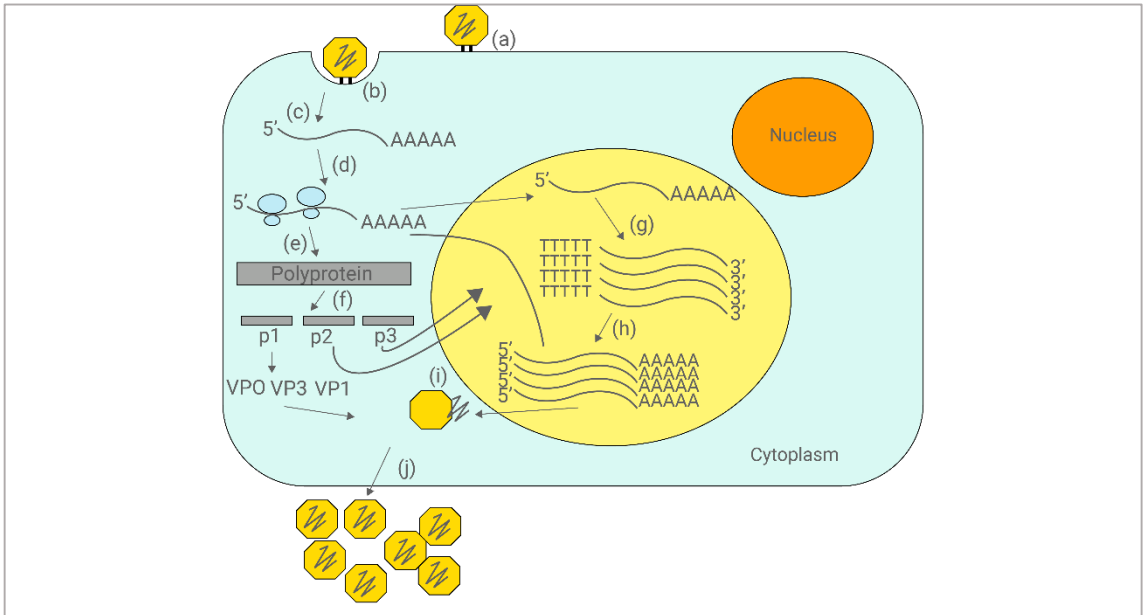

Figure 4: Schematic overview of HRV replication cycle. (a) Virus binds to cell receptor, (b) triggering endocytosis, and (c) genome uncoating. (d) The released positive-strand genome directs the immediate translation (e) of the polyprotein. (f) The nascent polyprotein is cleaved cotranslationally to produce the virus proteins. The RNA synthesis occurs anchored on vesicle membranes. (g) The positive genome is copied by the viral RNA polymerase to form the full-length negative RNA replicative intermediate, which then (h) serves as a template to produce additional positive RNA genomes. (i) The capsid proteins and the newly synthesized RNA genomes are assembled into virions, (j) which are released by cell lysis.

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sensitivity for human rhinovirus isolation from clinical specimens. Human rhinovirus shedding peaks around 48 h after infection and declines rapidly but may remain at low levels for up to 3 weeks. The optimal growth temperature for human rhinovirus is 33 C–35 C in a roller drum, and cultures should be examined for 10–14 days [33,34]. The presence of human rhinovirus is indicated by the typical CPE and can be confirmed by the acid sensitivity of the isolate. Unlike other picornaviruses, human rhinoviruses are acid-labile, a property that differentiates them from enteroviruses. Rapid assays for human rhinovirus detection, like immunofluorescence and other antigen detection methods, are not available because of many serotypes. The homotypic nature of human rhinovirus antibodies restricts serology to experimental settings. RT–PCR is the most sensitive method for human rhinovirus detection. Real-time PCR-based assays have been developed in various laboratories, mostly primers directed to the conserved 59UTR of the genome. They have the potential to detect serotypes of both human rhinovirus species. Real-time PCR multiplex assays are used to conserved sequences of different viral species and genera, but recently developed methods such as Mass-Tag and MultiCodePLx can detect several viral pathogens in a single run and are expected to become techniques of choice for large-scale sample testing soon.

Respiratory Adenoviruses

Adenoviruses are non-enveloped, icosahedral DNA viruses of the genus Mastadenovirus that belongs to the family Adenoviridae. The adenovirus capsid consists of three antigenically, morphologically, and functionally distinct types of capsomere: hexon, penton fibers, and penton bases. Penton fibers project from the penton bases (Figure 5).

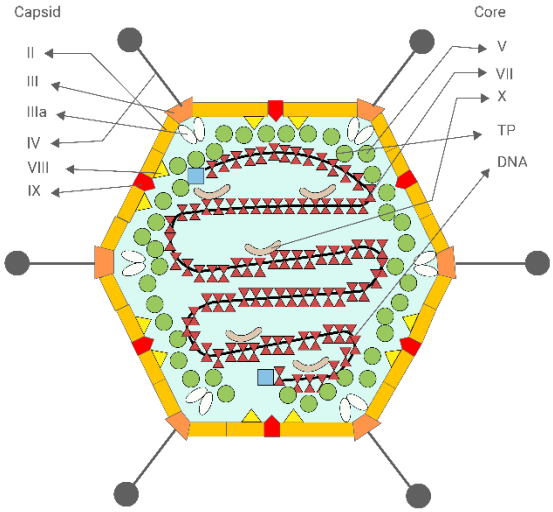

Figure 5: Schematic organization of the adenovirus virion. A naked icosahedral capsid contains a double-stranded DNA genome. The main capsid proteins are the hexon (II), penton base (III), and the penton fiber (IV), which projects from each vertex and binds to cell receptor. In addition to these, other capsid proteins are depicted (IIIa, VIII, and IX). The viral core contains the genome and the proteins V, VII, X, and TP – terminal protein).

The penton and hexon bases contain complement fixing, group-specific antigens common to all human adenoviruses, while the fibers have primarily hem agglutination-inhibiting and neutralizing, type-specific antigens. Serum neutralization allows the classification of human adenoviruses in 51 distinct serotypes, dispersed in six species, A–F. Usually, adenoviruses are accompanied by small, single-stranded DNA parvoviruses known

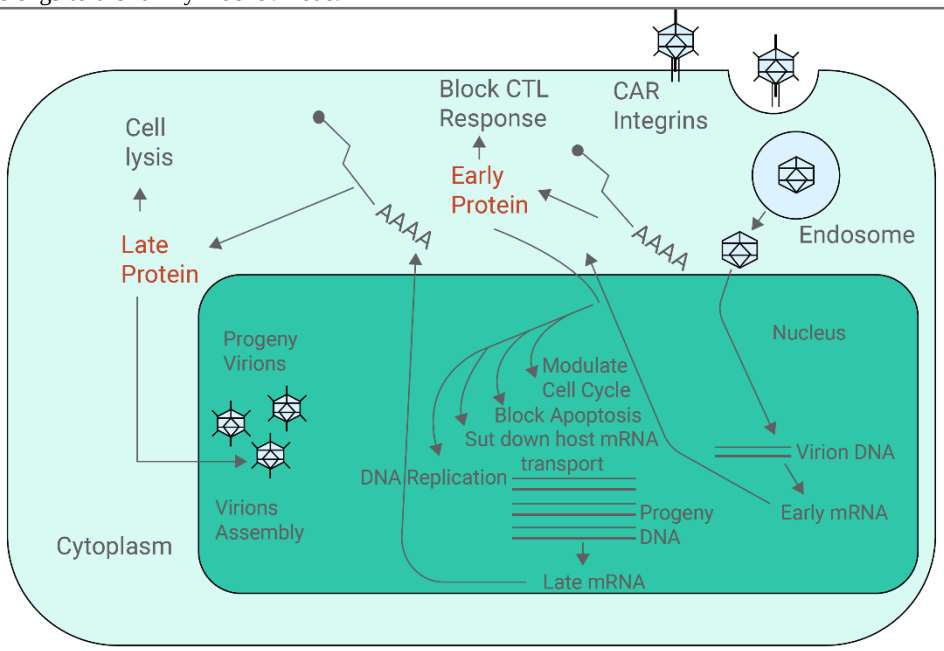

Figure 6: Adenovirus replication cycle. The virus binds to the receptor CAR (Coxsackie B and adenovirus receptor) and integrins. After adsorption, virus is internalized by receptor-mediated endocytosis and directed to the nuclear pore where final disassembly occurs. The viral DNA genome is released into the nucleus and the early set of genes are expressed. Early viral gene products mediate further viral gene expression and DNA replication. Then the late viral genes are expressed, generating structural proteins, and assembly of progeny virions occurs. New viruses are released by cell lysis.

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as adeno-associated viruses, which do not seem to cause any specific disease. The fiber protein attaches to the host cell through the protein Coxsackie B and adenovirus receptor (CAR), a protein of the immunoglobulin superfamily that serves as a high-affinity receptor for the binding of adenovirus species A, C, D, E, and F. CD46, a plasma membrane protein, is the ligand for the fiber of adenovirus species B. After the fiber or receptor binding, the interaction of the penton base with cell surface integrins triggers endocytosis. It exposes the virus to the cytosol, with microtubule-mediated transport to the nucleus and final break up at the nuclear pore. In the nucleus, a set of early mRNAs is transcribed to directly translate proteins that modulate the cell cycle, block apoptosis, cease the host mRNA transport from the nucleus and DNA replication [36]. While the 'late' mRNAs are transcribed, and their products include viral structural proteins. Virus assembly occurs in the nucleus, and the infectious cycle is finished by the release of about 1 million virions upon cell lysis (Figure 6).

Adenoviruses are stable over a wide pH range (5–9), resistant to ether, chloroform, and isopropyl alcohol. They are stable for weeks at room temperature and years at around 20°C or colder; they can even be lyophilized. Adenoviruses are inactivated by sodium hypochlorite and by exposure to 60°C for 2 min [37].

Diagnosis

Adenoviruses can be detected in respiratory, ocular, or ear secretions, but the clinical correlation is required because asymptomatic virus shedding is common. Isolation of adenoviruses in cell culture with identification by IF has been in use for decades. Still, direct detection of viral antigens or viral DNA by PCR in clinical samples is a sensitive and rapid alternative. Adenoviruses replicate well in continuous cell lines of epithelial origin, such as HEp-2, HeLa, and A549, and can be adapted to grow in human embryonic lung fibroblasts. In culture, they cause a characteristic CPE, and maintenance of cultures for two weeks combined with blind passage may increase adenovirus recovery. Inoculation of cells by centrifugation in the shell vial format followed by immunostaining may shorten the detection time. Rapid antigen detection by immunochromatography [38] is around 95% sensitive compared to cell culture and can be easily used in the point-of-care diagnosis of adenovirus. However, both conventional and real-time PCR is currently accepted as the most sensitive diagnostic methods. Positive results by PCR should be interpreted with caution, given the propensity of adenoviruses to cause latency. Quantitative real-time PCR can also be used to monitor viral load in transplant recipients or immunosuppressed patients, allowing for appropriate interventions to be initiated, such as immunosuppressive regimen adjustment. Several serologic tests can detect antibodies to the common hexon antigen [39,40]. However, their clinical utility is restricted.

Human Coronaviruses Unrelated to SARS

Based on antigenic and genetic studies, the known HCoVs are distributed in two of the three coronavirus groups so far identified. HCoV 229E and -NL63 belong to group I, HKU1, SARS-CoV, and HCoV-OC43 belong to group II, whereas group III contains no known human agents avian coronaviruses [41-45]. Coronaviruses are enveloped viruses with distinct virion morphology, widely spaced, long petal-shaped spikes at the surface that give out the virus a crownlike appearance, origin of the name corona (Figure 7).

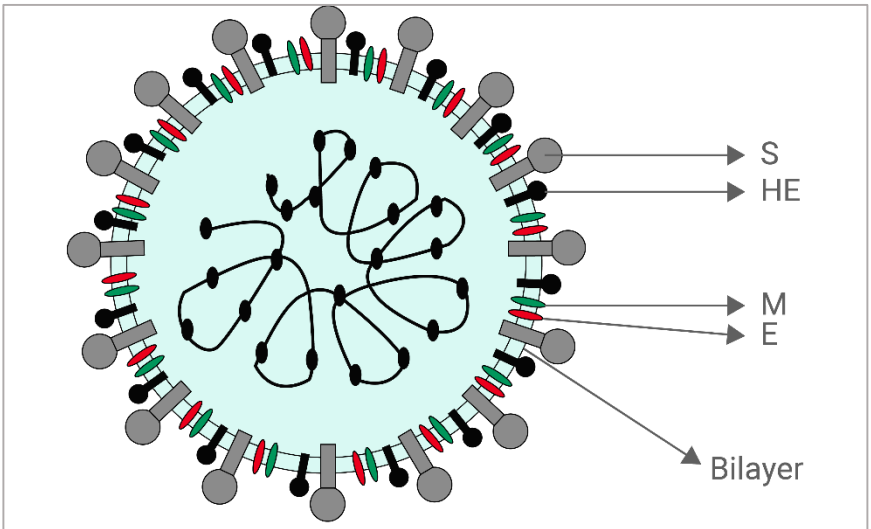

Figure 7: Schematic coronavirus structure. The virions are spherical, with an envelope containing a prominent crown ('corona') of peplomers of S (spike) glycoprotein. HE (hemagglutinin), E (small envelope protein), and M (membrane glycoprotein). The genome is a positive-stranded RNA associated with the N (nucleocapsid phosphoprotein), composing the helical RNP (ribonucleoprotein).

The viral envelope contains a long helical nucleocapsid with single-, positive-stranded RNA 27–32 kb in size, the largest known viral-RNA genome. Coronavirus RNA synthesis happens in the cytoplasm through a negative-strand RNA intermediate.

The viral RNA is capped at the 59 end, where a UTR follows a 'leader' sequence. At the 39 end, there is a terminal UTR, followed by a poly (A) tail [46]. The ORF 1 of the genomic RNA is translated into a polyprotein that is processed to yield the transcriptase–helicase complex proteins. The genomic RNA can be used as a template to synthesize negative-sense RNAs, which

are used to synthesize full-length genomic RNA and subgenomic mRNAs. The mRNAs direct translation of the viral structural and nonstructural proteins. Progeny viruses assemble and bud in vesicles between the endoplasmic reticulum and the Golgi apparatus, later released by exocytosis. The viral envelope contains the structural proteins S (spike), M (membrane), E (envelope), and only in the case of some group II coronaviruses, the HE. The S glycoprotein contains neutralizing and T cell epitopes and functions as the cell receptor ligand. The M protein is embedded in the envelope and interacts with the N

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(nucleocapsid) protein during maturation. In addition to the nucleocapsid and envelope proteins, a replica is present in cells infected by all coronaviruses [46-49]. Except for HCoV-OC43, which binds the cell receptor via the HE, the other HCoVs bind via the S (spike) protein. The S protein of HCoV-229E binds to the metalloprotease human aminopeptidase N, or CD13, at the cell surface, and entry is independent of the enzymatic activity of the receptor. The HE of HCoV-OC43 binds to the sialic acid present in glycoproteins on the cell surface, and this interaction facilitates infection. HCoV-NL63, like SARS-CoV, utilizes the angiotensin-converting enzyme 2 (ACE2) as a cell receptor.

Diagnosis

Laboratory diagnosis of HCoVs in clinical samples by isolation in cell cultures is challenging. RT–PCR-based assays for HCoVs utilizing primers for relatively conserved genome regions are currently the best alternative for virus detection. More recently, quantitative real-time PCR for HCoVs has been developed in several laboratories, providing a fast and sensitive way to detect and determine the viral load, with potential applications in pathogenesis studies. Serologic diagnosis of HCoVs is sensitive and specific but has limited application in case management.

SARS Coronavirus

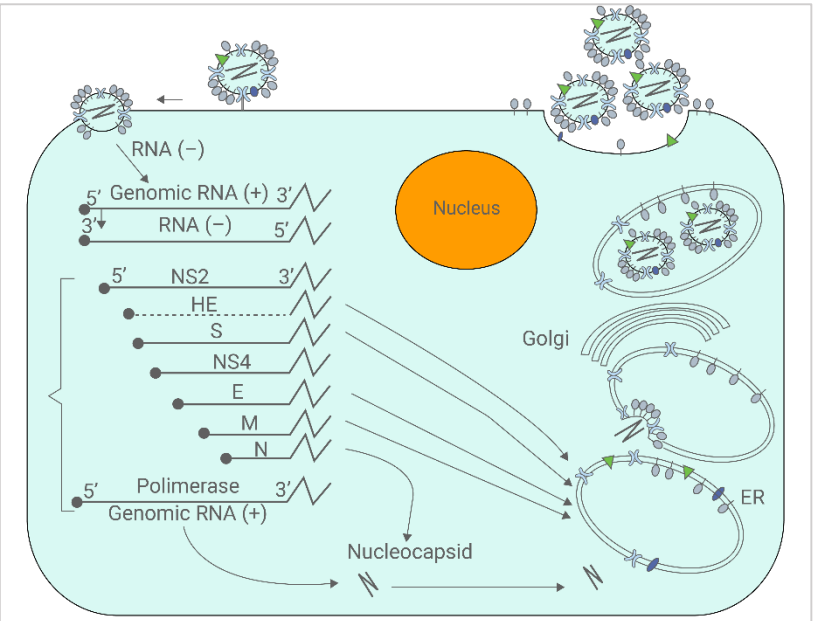

Figure 8: Schematic representation of the life cycle of a coronavirus. Virion binds receptors on the plasma membrane by the S glycoproteins resulting in fusion of viral envelope and plasma or endosomal membrane. ORF 1 of the genomic RNA is translated generating the RNA-dependent RNA polymerase that synthesizes negative-sense RNAs, which serve as templates to generate full-length genomic RNA and subgenomic (NS2, HE, S, NS4, E, M, and N). mRNAs are translated in structural and nonstructural proteins. The N protein and newly synthesized genomic RNA form the nucleocapsid. Other structural proteins are inserted in the endoplasmic reticulum (ER) where they are cotranslationally glycosylated and trimerized. The nucleocapsids are then enclosed by these proteins in the ER and transported to the Golgi apparatus. Mature virions are apparently released by exocytosis-like fusion of smooth-walled vesicles with the plasma membrane.

SARS-Coronavirus shares structural features and genome organization with other Coronaviridae [47]. The identification of the peculiar coronavirus morphology by electron microscopy of Vero E6 cells injected with oropharyngeal material from a patient was the initial clue to identifying the agent. The viral genome is 29 727 nt in length, with 14 ORFs coding for 28 putative proteins. It is known that SARS-CoV nsp1 protein increases cellular RNA degradation and thus might facilitate SARS-CoV replication or block immune responses. Trimers of the S protein form the peplomers and bind the cell receptor, being the primary target for neutralizing antibodies. M and N proteins of SARS-CoV can induce apoptosis of host cells and play an essential role in pathogenesis. SARS-CoV is phylogenetically different from previously known coronaviruses, but isolates from different origins are relatively similar. Genome analysis reveals that SARS-Coronavirus is neither a host-range mutant nor a recombinant of earlier known coronaviruses but rather an independently emerged virus. SARS-Coronavirus seems to have evolved from an animal SARS-like virus, acquiring more excellent fitness in humans during the outbreaks, probably through the appearance of nucleotide deletions in ORF8. During the infection, the N-terminal portion of the spike glycoprotein binds to the virus receptor, identified as the metallopeptidase ACE2 homolog, triggering pH-dependent endocytosis [47-48]. SARS-CoV spike protein can also bind the dendritic cell-specific C-type lectin ICAM-3 grabbing nonintegrin (DC-SIGN), which does not result in dendritic cell infection by the agent but allows for SARS-CoV to be transported to susceptible target cells elsewhere. Upon entry, the SARS-CoV replication cycle is probably similar to that of other coronaviruses.

Diagnosis

The ability to grow SARS-CoV in Vero E6 cell cultures was critical to identifying the agent. SARS-CoV can be recovered by isolation from respiratory secretions, feces, and urine. Still, this procedure can only be done in biosafety level 3 laboratories not available in most world hospitals. Although not useful for early diagnosis, seroconversion determined by IFA or EIA remains the gold standard for confirming SARS diagnosis. IgG seroconversion is detectable in over 90% of patients at around day 28. Rapid diagnosis by antigen detection is feasible but less sensitive than RT–PCR. Real-time quantitative RT– PCR of nasopharyngeal aspirates is the method of choice with around 80% sensitivity. Because of low viral loads in the upper respiratory tract in the first few days of illness, SARS-CoV is detectable by RT–PCR in nasopharyngeal aspirates in only one-third of patients at presentation and two-thirds just at day 14. RT–PCR may be favorable for SARS-CoV in stools from as much as 97% of patients at day 14 and in urine in 42% of samples at day 15. The sensitivity of RT–PCR could be

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increased by testing multiple specimens, including nasopharyngeal, serum, and fecal samples.

Human Bocavirus (HBoV)

Human bocavirus was recently discovered using an elegant metagenomic approach and has now been detected in samples from children with ARI in studies worldwide. Human bocavirus was described in 2005 after screening a pool of clinical respiratory samples that had tested negative for other viral agents.

- Bioinformatic analysis of the sequences revealed it to be a new member of the family Parvoviridae, provisionally categorized by sequence homology and genome organization in the genus Bocavirus, which already includes two other viruses: the canine minute virus and bovine parvovirus-1.
- Human bocavirus
- virions consist of small non-enveloped icosahedral particles with a single-stranded DNA genome of about 5300 nt, arranged in a way similar to that of other known bocaviruses, with three ORFs. Of the three ORFs, two encodes the nonstructural proteins NS1 and NP-1, the third nesting the two capsid proteins VP1 and VP2 (Figure 9).

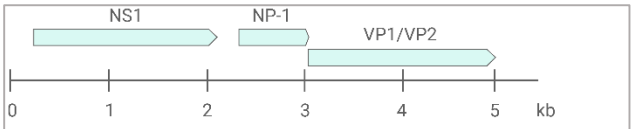

Figure 9: Schematic map of HBoV genome. The single-stranded DNA genome contains three ORFs: NS1, NP-1, and VP1/VP2. VP1 and VP2 are alternative splicing products of the same ORF.

NS1 is the viral polymerase, and the function is still unknown. The human bocavirus replication cycle is similar to that of other autonomous Parvoviridae [48]. Two genetically distinct clusters have been detected, and the degree of variability is greater in the gene of capsid proteins. Human bocavirus has not been propagated in cell cultures, and this has hampered cell biology studies and the production of virus stocks for pathogenesis studies.

Diagnosis

The diagnostic method available for HBoV is PCR on nasopharyngeal aspirates, swabs, and washes. Primers conducted to the more conserved NS1 gene are recommended. Besides its diagnostic importance, real-time PCR assays can quantitate viral loads. Therefore, it can contribute to HBoV pathogenesis investigation. Serology has been described using recombinant VP2 and baculovirus-expressed VP2 VLPs as antigens, but as for other respiratory viruses, its application to case management would be limited [49].

Conclusion

Current and future diagnostic options will include antigen, molecular, and culture-based methods. The performance characteristics and limitations of these methods will vary significantly with the new generations of assays. Diagnostic virologists and clinicians must understand these characteristics and limitations.

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Ethical consideration: Not applicable

Source of funding: Self

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