Enteroviruses and Parechoviruses

JAMES J. DUNN

1Department of Pathology, Texas Children’s Hospital, Houston, TX 77030

ABSTRACT Infections with enteroviruses and human parechoviruses are highly prevalent, particularly in neonates, where they may cause substantial morbidity and mortality. Individuals with B-cell-related immunodeficiencies are at risk for severe enteroviral infections, usually a chronic and fatal meningoencephalitis. In transplant recipients and patients with malignancy, enterovirus infections typically involve the respiratory tract, but cases of severe, disseminated infection have been described. The mainstay of diagnosis for enterovirus and human parechovirus infections involves the use of molecular diagnostic techniques. However, routine nucleic acid-detection methods for enteroviruses will not detect human parechoviruses. Laboratory diagnosis of these viral infections is important in determining a patient’s prognosis and guiding clinical management.

CLASSIFICATION AND BIOLOGY

Human enteroviruses (EVs) and parechoviruses (HPeV) belong to the family Picornaviridae (1). The human enteroviruses are classified into four species based on molecular and biologic characteristics: human enterovirus A (HEV A), HEV B, HEV C, and HEV D, with the traditional names retained for individual serotypes (Table 1). Genetic characterization of two echovirus serotypes, 22 and 23, has resulted in their reclassification into a separate new genus, Parechovirus, and they are termed HPeV 1 and 2, respectively. Subsequent studies have demonstrated that there are now 16 distinct HPeV types, of which HPeV 1–6 are known to cause infections in humans.

EVs and HPeVs are small (approximately 300 Å in diameter) nonenveloped viruses with a positive-sense, single-stranded RNA genome of approximately 7,400 nucleotides. A single open reading frame encoding approximately 2,185 amino acids is flanked 5’ and 3’ by nontranslated regions of approximately 750 and 100 nucleotides, respectively. The coding region is subdivided into the following three regions (5’ to 3’): the P1 region encodes the structural proteins (VP4, VP2, VP3, and VP1 for EV and VP0, VP3, and VP1 for HPeV), and the P2 and P3 regions encode the nonstructural proteins, such as the RNA-dependent RNA polymerase, proteases, and other proteins necessary for intracellular replication. The entire polyprotein is cleaved co- and posttranslationally by the virally encoded proteases to generate the gene products (11 for EV, 10 for HPeV) (1).

EPIDEMIOLOGY AND CLINICAL MANIFESTATIONS IN IMMUNOCOMPETENT HOSTS

EV and HPeV infections occur worldwide, although rates of infections may vary noticeably by location, and are seasonal in nature. Individuals living in temperate climates experience considerably higher rates of EV infection in the summer and fall months. HPeV incidence has been reported to show a seasonal pattern in temperate climates, with different types co-circulating simultaneously. In the United States from 1970 to 2005, EV infections occurring from June through October accounted for approximately 78% of reports (2). In tropical and subtropical areas, transmission occurs throughout the year. In the U.S., an estimated 10 million to 15 million symptomatic nonpolio EV infections occur each year (3). The predominant EV serotypes produce endemic disease with various periodicities and in patterns that vary...
TABLE 1 Human enteroviral species and serotypes

<table>
<thead>
<tr>
<th>Human enterovirus species</th>
<th>Serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEV A</td>
<td>Coxsackievirus A2–A8, A10, A12, A14, A16</td>
</tr>
<tr>
<td></td>
<td>Enterovirus 71, 76, 89–92, 114, 119, 120, 121</td>
</tr>
<tr>
<td>HEV B</td>
<td>Coxsackievirus A9, B1–6</td>
</tr>
<tr>
<td></td>
<td>Echovirus 1–9, 11–21, 24–27, 29–33</td>
</tr>
<tr>
<td></td>
<td>Enterovirus 69, 73–75, 77–88, 93, 97, 98, 100, 101, 106, 107, 110</td>
</tr>
<tr>
<td>HEV C</td>
<td>Coxsackievirus A1, A11, A13, A17, A19–A22, A24</td>
</tr>
<tr>
<td></td>
<td>Poliovirus 1–3</td>
</tr>
<tr>
<td></td>
<td>Enterovirus 95, 96, 99, 102, 104, 105, 109, 113, 116–118</td>
</tr>
<tr>
<td>HEV D</td>
<td>Enterovirus 68, 70, 94, 111, 120</td>
</tr>
</tbody>
</table>

regionally, a feature likely due to the availability of susceptible host populations within a community. Although EV and HPeV infections occur in all age groups, it is generally infants and young children that have the highest rates of infection and disease (2).

Human EV and HPeV infections are acquired by contact with the virus, which is shed in the feces or upper respiratory tract of direct and indirect contacts (4). The incubation period for enteroviral infections may vary according to the clinical syndrome, but is generally 7 to 14 days (range, 3 to 35 days). Following primary infection, EV can be shed in the upper respiratory tract for 1 to 3 weeks and in the feces for several weeks to months. EV excretion does not necessarily imply association with disease, because most such excretion is asymptomatic. Reinfection with the same serotype can occur despite previous immunity.

In immunocompetent hosts, nonpoliovirus EVs are responsible for numerous types of infections that result in a broad spectrum of clinical manifestations, from asymptomatic or mild self-limiting disease to severe, disseminated disease that is sometimes fatal. The most common symptomatic manifestation of EV infection is an acute, nonfocal febrile illness affecting mainly infants of less than 1 year of age (5–8). EVs may cause upper respiratory tract symptoms alone or with fever, but demonstrating the clinical significance of infection has been complicated by the occasional isolation of these viruses from healthy individuals with no apparent clinical evidence of disease (9). Involvement of the lower respiratory tract may cause exacerbations of asthma, chronic bronchitis, laryngotracheobronchitis, and less commonly, bronchiolitis and pneumonia.

Herpangina, characterized by painful vesicular lesions on the soft palate, uvula, tonsils, and posterior pharynx, is seen most commonly in children 1 to 7 years of age and is often accompanied by fever, sore throat, dysphagia, and malaise (10). Hand-foot-and-mouth disease is characterized by vesicular stomatitis and cutaneous lesions on the distal ends of the extremities and is commonly associated with coxsackievirus A16 and EV 71. Outbreaks of hand-foot-and-mouth disease caused by EV 71 have been associated with serious central nervous system (CNS) disease, including acute motor-neuron disease and brainstem encephalitis (11, 12). Several EV serotypes, particularly EV 70 and coxsackievirus A24, are known to cause acute conjunctivitis, keratoconjunctivitis, and hemorrhagic conjunctivitis (13). EV-D68 has emerged as a significant respiratory pathogen, primarily in pediatric patients, often requiring treatment in an intensive-care setting (14).

The foremost EV CNS infections include aseptic meningitis, encephalitis, poliomyelitis, and poliomyelitis-like illness (15). Nonpolio EV infections have also been implicated in cases of Guillain-Barré syndrome, acute transverse myelitis, and cerebellar ataxia (16–18). A number of myotropic diseases have been ascribed to the EVs. These include pleurodynia (Bornholm disease), acute myocarditis, chronic dilated cardiomyopathy, and myositis (15).

Since the introduction of poliovirus vaccines in 1955, endemic poliovirus infections have largely been controlled, and the region of the Americas has been certified as eradicated of wild-type polioviruses since 1994 (19). However, certain underdeveloped parts of the world continue to have endemic poliovirus infections (20). Sporadic cases and outbreaks of paralytic poliomyelitis due to strains contained in the live, attenuated oral poliovirus vaccine (OPV) continue to occur in many parts of the world (21). The incidence of vaccine-associated paralytic poliomyelitis (VAPP) is approximately 1 case per 2.9 million doses of vaccine distributed, and it is associated with the first dose more often than with subsequent doses. Since the policy change to exclusive use of inactivated poliovirus vaccine (IPV) in 2000, no cases of VAPP have occurred in the United States.

HPeV1 and HPeV2 have been associated with mild gastrointestinal and respiratory symptoms; more serious diseases have been occasionally reported, including myocarditis, encephalitis, pneumonia, meningitis, flaccid paralysis, and fatal neonatal infection (22). HPeV3 has been associated with more severe clinical manifestations in the form of sepsis-like and CNS illnesses, particularly in neonates and infants (23, 24). HPeV3 infections are uncommon in subjects older than 10 years of age. HPeV types 4–8 seem to cause disease similar to those associated with HPeV1 and HPeV2 infections (25).
Protective immunity to EV infection, and likely HPeV, is serotype specific. In the well-studied poliovirus model, serum neutralizing antibody develops about 1 week after infection and persists for life. Although both humoral and cell-mediated immune response mechanisms occur following EV infection, it is the presence of serotype-specific antibody that is most important in limiting disease and eradicating the virus (4). Antibodies to the more commonly circulating EV serotypes are seen in 30% to 80% of individuals by adulthood (13). Transplacental transfer of maternal antibody effectively prevents or ameliorates EV disease in the infant (26–28). Most children less than 5 years of age have antibodies against HPeV1 and HPeV2, but seroprevalence for HPeV3 is quite low even into adulthood (29).

**HUMAN ENTEROVIRUS AND PARECHOVIRUS INFECTIONS IN IMMUNOCOMPROMISED HOST POPULATIONS**

Infections with EV or HPeV occur infrequently in immunocompromised individuals, but those with B-cell-related deficiencies are at particularly high risk for severe enteroviral infection, usually a chronic and fatal meningoencephalitis. Neonatal infection with EV or HPeV can cause substantial morbidity and mortality. In transplant recipients and patients with malignancy, EV infections typically involve the respiratory tract, but cases of severe, disseminated infection have been described.

**Primary B-Cell-Associated Immunodeficiencies**

Patients with congenital isolated B-cell deficiency or combined immunodeficiencies are at considerable risk of infection. Among hereditary B-cell-associated immunodeficiency syndromes, X-linked agammaglobulinemia (XLA), common variable and severe combined immunodeficiency (SCID) syndromes, and X-linked hyperimmunoglobulin M (hyper-IgM) (XHIM) syndrome have been associated with persistent progressive EV infection. Individuals with congenital immunodeficiency have approximately 3,000 times the risk of VAPP as do normal children (30). Antibody-deficient individuals infected with EV may develop chronic meningitis or meningoencephalitis lasting many years, often with a fatal outcome (31–33) (Table 2). However, with the availability of intravenous immunoglobulin (IVIG) preparations and antipicornaviral drugs and the early recognition of EV infection, fewer of these patients appear to be progressing to the classical description of chronic EV meningoencephalitis of agammaglobulinemia (CEMA).

**TABLE 2** Clinical syndromes associated with EV and HPeV infection in immunocompromised hosts and reported serotypes

<table>
<thead>
<tr>
<th>Patient population</th>
<th>Clinical manifestations</th>
<th>Enterovirus serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congenital B-cell deficiency</td>
<td>Chronic meningoencephalitis</td>
<td>Echovirus 2, 3, 5, 6, 7, 9, 11, 13–15, 17–19, 21, 24, 25–27, 29, 30, 33</td>
</tr>
<tr>
<td></td>
<td>Dermatomyositis-like syndrome</td>
<td>Coxsackievirus A4, A11, A15, B2, B3</td>
</tr>
<tr>
<td></td>
<td>VAPP</td>
<td>Echovirus 2, 3, 5, 7, 9, 11, 17, 19, 24, 30, 33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coxsackievirus B3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Poliovirus 1, 2, 3</td>
</tr>
<tr>
<td>Transplant recipients</td>
<td>URI, LRTI, ARDS</td>
<td>Echovirus 6, 11, Coxsackievirus A9, B (untyped)</td>
</tr>
<tr>
<td></td>
<td>Meningoencephalitis</td>
<td>Echovirus 11, 18, 19, Coxsackievirus A9</td>
</tr>
<tr>
<td></td>
<td>Myocarditis/pericarditis</td>
<td>Echovirus 11, Coxsackievirus B3</td>
</tr>
<tr>
<td></td>
<td>Acute flaccid paralysis</td>
<td>Echovirus 19</td>
</tr>
<tr>
<td></td>
<td>Gastroenteritis</td>
<td>Echovirus (untyped); Coxsackievirus A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coxsackievirus B3–B5; EV71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Echovirus 11, Coxsackievirus B3</td>
</tr>
<tr>
<td>Malignancy</td>
<td>Meningoencephalitis</td>
<td>Coxsackievirus B3–B5</td>
</tr>
<tr>
<td></td>
<td>URI, LRTI</td>
<td>Echovirus 11, 13, 20, Coxsackievirus B3–B5; EV71</td>
</tr>
<tr>
<td></td>
<td>Myocarditis</td>
<td>Echovirus 11</td>
</tr>
<tr>
<td>Neonates</td>
<td>Meningitis/encephalitis</td>
<td>Coxsackievirus B3</td>
</tr>
<tr>
<td></td>
<td>Pneumonia</td>
<td>Echovirus 4, 6, 7, 9, 11, 17, 19, 30, Coxsackievirus B1–B5; HPeV1, 3</td>
</tr>
<tr>
<td></td>
<td>Hepatitis</td>
<td>Echovirus 6, 7, 9, 11, 17, 19, 20, Coxsackievirus B3; HPeV1</td>
</tr>
<tr>
<td></td>
<td>Myocarditis</td>
<td>Echovirus 4, 6, 7, 9, 11, 14, 17, 19, 20, 21; Coxsackievirus B1–B4</td>
</tr>
<tr>
<td></td>
<td>DIC</td>
<td>Echovirus 7, 11; Coxsackievirus B1–B5</td>
</tr>
<tr>
<td></td>
<td>Sepsis-like syndrome</td>
<td>Echovirus 4, 6, 11, 17; Coxsackievirus B1–B4</td>
</tr>
<tr>
<td>HIV</td>
<td>URI, LRTI</td>
<td>HPeV1, 3, 4</td>
</tr>
<tr>
<td></td>
<td>Meningoencephalitis</td>
<td>Echovirus 33</td>
</tr>
<tr>
<td></td>
<td>VAPP</td>
<td>Echovirus 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Poliovirus 2</td>
</tr>
</tbody>
</table>

*VAPP, vaccine-associated paralytic poliomyelitis; URI, upper respiratory-tract infection; LRTI, lower respiratory-tract infection; ARDS, acute respiratory-distress syndrome; DIC, disseminated intravascular coagulation.*
XLA is characterized by a block in B-cell development at the pre-B-cell stage, typically resulting in markedly decreased levels or an absence of both mature B cells and immunoglobulin production in affected male subjects. Because IgG is actively transported across the placenta, affected newborns have normal levels of serum IgG at birth and few, if any, symptoms. As the maternally derived antibodies are catabolized, serum IgG gradually decreases, and since immunoglobulin production is impaired in XLA, severe hypogammaglobulinemia and increased susceptibility to infection develop. Thus, many patients with XLA are asymptomatic for the first few months of life and begin to have recurrent infections between 4 and 12 months of age; a smaller percentage of patients will present after the first year of life (34).

Bacterial respiratory-tract infections are the most common clinical manifestations of XLA and resistance to viral infections is generally intact, except for infections caused by EVs. Because antibodies are important in neutralizing these viruses during their passage through the bloodstream, patients with XLA lack an important mechanism of resistance to the EVs. Historically, when OPV was in common use, some patients with XLA would develop VAPP after immunization (31, 35). A U.S. registry of 201 patients with XLA identified enteroviral infections in 10 individuals, the vast majority of whom had chronic meningoencephalitis (34).

XHIM syndrome is a rare cause of immunodeficiency characterized by normal or elevated levels of IgM and low levels of IgG, IgA, and IgE. Clinical manifestations include neutropenia, cholangitis, tumors, and frequent infections with bacteria, *Pneumocystis jirovecii*, *Candida* spp., and cryptosporidium. In XHIM, normal B-cell function is disrupted resulting in abnormal antibody responses to T-cell-dependent antigens, a failure to switch from IgM to other immunoglobulin isotypes, a lack of immunologic memory, and an inability to form germinal centers in lymph nodes. Meningitis, encephalitis, and encephalopathy have been reported as complications in up to 12% of patients with XHIM syndrome (36). In one series of patients with XHIM syndrome, three had EV meningoencephalitis despite IVIG treatment yielding therapeutic serum immunoglobulin levels (37).

CEMA is a disease marked by prolonged enteroviral infection of the CNS. Patients may present with slowly progressive neurologic symptoms or more acutely with fever, headache, and seizures. Once CEMA is recognized, its progression can generally be slowed by the administration of IVIG, although most patients continue to have episodic relapses. Some patients develop a dermatomyositis-like syndrome with “woody” edema of their extremities, erythematous rash, and evidence of fasciitis and myositis on biopsy material. EVs can occasionally be isolated from skin, muscle, or liver biopsies (32, 38). Prior to the use of current treatment modalities, neurologic manifestations of EV infection associated with congenital B-cell immunodeficiency included paralysis/paresis, seizures, long-term cognitive impairment, developmental regression, and coma (31).

At the onset of disease, viral cultures of CSF from these patients may be negative and remain so for long periods. Molecular methods may show that culture-negative CSF samples contain EV, even in the presence of normal cell counts and protein levels (39, 40). EV may persist in the CNS, where levels of passively transferred antibody are low. The CNS is then the site from which the virus may spread secondarily to other organs and tissues. The most commonly isolated EVs are echoviruses (primarily echovirus 11), followed by polioviruses and coxsackieviruses (31, 32) (Table 2). On rare occasions, reverse transcription-PCR (RT-PCR) results may intermittently be negative during the course of CEMA (39–42). Virus is isolated only rarely from the stools of patients with CEMA, except during the disseminated phases of infection or in those infected with poliovirus strains. A negative RT-PCR result from CSF does not necessarily exclude the diagnosis, and subsequent testing of an additional CSF specimen would be warranted if enteroviral infection is suspected.

### Transplant Recipients

Opportunistic viral infections occur frequently in patients undergoing bone marrow transplantation and can be a major cause of morbidity and mortality. The main group of viruses responsible for infection after transplantation is the herpesviruses, which may either be transmitted by the graft or cause a clinically manifest reactivation of infection. Certainly, given the trend for more aggressive immunosuppressive therapy both pre- and post-transplant by use of B-cell-depleting antibodies, the incidence and severity of EV infections may increase among transplant recipients. Moreover, manipulation of hematopoietic stem cell grafts (e.g., CD34 selection) can further delay already impaired B-cell reconstitution and place patients at risk for enteroviral infections during their immune-recovery phases. Ideally, patients with active EV infections should have documented clearance of disease prior to proceeding to transplant.

Several case reports of fatal disseminated EV infections have been reported for bone-marrow-transplant (BMT) recipients (43–48) (Table 2). Initial signs of in-
fection were apparent from 12 to 53 days posttransplant and included clinical syndromes, such as encephalitis, myocarditis, pericarditis, pneumonia, and severe systemic infection. Most fatal cases among BMT recipients were those with unrelated donors. Reports of infectious gastroenteritis caused by coxsackieviruses among BMT recipients have also been associated with significant mortality (49, 50). Recovery from severe EV infection following bone-marrow or solid-organ transplantation has been described, even in cases with extensive CNS involvement (46, 51, 52).

Lung-transplant recipients are at increased risk for severe complications associated with respiratory infections; however, the incidence of EV-associated respiratory infections in these patients appears to be lower than those caused by more common respiratory viruses (53, 54). Respiratory illnesses in BMT recipients are confined mostly to the upper respiratory tract and result in mild to moderate respiratory disease, with clinical improvement within 1 to 2 weeks (55–57). Chakrabarti et al. (55) identified symptomatic EV upper respiratory-tract infections in 7 of 64 adult patients who had received T-cell-depleted allogeneic stem-cell transplants. Upper respiratory symptoms attributable to EV infection can be seen as early as 25 days after transplant, but may not appear for more than a year (58). On the other hand, lower respiratory-tract EV infection may be more severe. In stem-cell-transplant recipients with suspected lower respiratory-tract infections, EVs were isolated from 38% of BAL samples over an 8-year period (59). Of seven stem-cell-transplant recipients with radiographic evidence of lower respiratory-tract disease due to EV, five developed acute respiratory-distress syndrome (ARDS), three required mechanical ventilation, and three died (59, 60). In the fatal cases, the median onset of respiratory illness was 120 days after transplant (range, 37 to 160), and all patients either did not have engraftment or were being treated for relapse of leukemia/lymphoma. A fatal outcome after ARDS and subsequent respiratory failure due to EV infection has also been described for a child after stem-cell transplantation (48).

Starlin et al. (61) reported the first published case of acute flaccid paralysis due to a nonpoliovirus EV (echovirus 19) in a solid-organ-transplant recipient. This patient, who had cystic fibrosis, had undergone two bilateral lung transplants and a renal transplant and was undergoing intensive immunosuppressive therapy. The patient was treated with IVIG and pleconaril, with some objective clinical improvement in neurologic function. Subsequent cultures for EV were negative, although the patient succumbed to bacterial pneumonia and fungal sepsis.

Malignancy
EVs have occasionally been implicated in serious infections on oncology patients. In a 5-year study of pediatric patients with malignancy conducted in Greece, EV infections were documented for 55 patients with infection-related mortality of 14.5% (62). Of 77 children with acute leukemia, 43 (56%) developed documented enteroviral infection, whereas 5 of 37 (13%) patients with lymphoma and 7 of 236 (3%) patients with solid tumors developed EV infection. Severe manifestations, including encephalitis and myocarditis, were noted in 20% of those infected. In a prospective study of febrile episodes in 66 pediatric oncology patients, EVs were identified in two patients; one from a respiratory infection concomitantly with rhinovirus and varicella-zoster virus and the other from stool (63). EVs were identified in 4.7% of 105 viral infections in pediatric patients being treated for leukemia (64). Outbreaks of EV infection in oncology wards have also been described (49, 65).

Viral pneumonia is a frequent complication in patients undergoing intensive chemotherapy for treatment of hematologic malignancies. The significance of EVs in lower respiratory-tract disease was examined in one study in which EVs were isolated from three and five BAL specimens from patients with hematologic malignancies and recipients of stem-cell transplants, respectively (66). All patients with EV infection presented with fever at diagnosis, and 75% had pneumonia. Five patients subsequently died from pneumonia, although three of them had concomitant viral and/or bacterial infections. In adult patients with hematologic malignancies, six patients with EV-associated pneumonia were identified over a 5-year period, with one being fatal (67). A case of hand-foot-and-mouth disease in a patient undergoing chemotherapy for Ewing’s sarcoma has also been described (68).

EV meningoencephalitis and disseminated infection have been described for patients undergoing chemotherapy for hematologic malignancy or solid tumors (62, 69–74) (Table 2). In these patients, manifestations of EV infection included meningoencephalitis lasting at least 1 month, myositis, pneumonia, myocarditis, and hemorrhagic syndrome. Hypogammaglobulinemia and/or leukopenia were reported for the vast majority of these patients. While most patients survived infection with nearly complete resolution of neurologic function, several fatalities attributed to disseminated EV infection have been reported for pediatric patients with hematologic malignancies (62).

An outbreak of EV infection in a 2-month period (November and December) among five pediatric patients
with acute lymphoblastic leukemia and immunosuppression resulted in two deaths, one with chronic encephalitis and one with acute brainstem encephalitis (65). The three patients who recovered were in the maintenance phase of chemotherapy, while the two who died were in the induction phase.

Treatment of hematological malignancies with the chimeric anti-CD20 monoclonal antibody (mAb) rituximab induces rapid and long-lasting depletion of circulating B cells. Rituximab administration is associated with profound antibody deficiency of usually 6 to 12 months duration. The occurrence of EV meningoencephalitis following rituximab therapy has been reported for a child with immune thrombocytopenia and five adults with B-cell lymphomas (75–79). The time between rituximab treatment and neurologic manifestations was variable in each case, ranging from symptoms appearing during therapy to 11 months after completion.

**Neonates**

EV and HPeV infections in the newborn period are common. In population-based studies, enteroviral infections were found in 4% to 26% of hospitalized febrile infants younger than 3 months of age being evaluated for sepsis (5, 7, 80). In the summer and fall months, EV infections at less than 3 months of age accounted for 13% to 65% of hospital admissions (6, 8). Data reported to the National Enterovirus Surveillance System from 1983 to 2003 revealed that 11.5% of reported neonatal infections with known outcome resulted in death, and infection with coxsackievirus B4 was associated with a significantly higher risk of mortality than infection with other EV serotypes (81) (Table 2). In 2007 and 2008, coxsackievirus B1 was associated with severe neonatal disease, myocarditis, and several infant deaths (82, 83). Reports of case fatality rates for neonatal EV infections have been as high as 83% (27, 84, 85). Mortality from HPeV in the neonatal period is not well described.

Transmission of EV and HPeV from mother to infant is relatively common and may occur transplacentally or through contact with maternal secretions during vaginal delivery, blood, or upper respiratory-tract secretions. Transmission of EV at the time of birth appears to be the most common route of exposure, occurring in 30% to 50% of mothers with seroconversion in pregnancy (28, 86). Reports of onset of symptoms in the first two days of life and detection in CSF on day 2 of life suggest that in utero HPeV transmission occurs (87). EVs have been isolated from amniotic fluid obtained during the third trimester via amniocentesis, despite the presence of intact membranes, suggesting that the virus may also be transmitted transplacentally (88, 89). Maternal viral illness within 2 weeks prior to delivery, with symptoms such as fever, respiratory-tract infection, or abdominal pain, has been reported for 59% to 68% of infected neonates (90). Premature infants are particularly vulnerable to infection due to the limited transplacental transfer of maternal immunoglobulins, low IgM and IgA production, deficiency of complement components, and impaired cellular function (91). It is likely that transplacentally acquired maternal antibody has an important role in modifying perinatal infection in the newborn (92). This passively acquired specific antibody is lacking in infants whose mothers become infected within the last few days of pregnancy. Infection of the placenta has been demonstrated in cases of fetal or neonatal death or severe neonatal morbidity (93–95).

These EV infections are often seen in term neonates who have no perinatal risk factors for sepsis and who have been discharged in seemingly good health after birth. The manifestations of neonatal EV disease range from unapparent infection to overwhelming severe, life-threatening disease. Clinical manifestations of EV infection can develop as early as day 1 of life and throughout the neonatal period. The majority of neonates experience asymptomatic infection or benign illness without sequelae. Early signs and symptoms of newborn infection may be nonspecific. In those with mild disease, fever and other symptoms typically regress within a week. Severe illness develops in a minority of infected newborns, resulting in high rates of mortality and morbidity because multiple organ systems, such as the CNS, lungs, liver, and heart, are commonly involved (Table 2). Severe infant disease is correlated with prematurity, male sex, and onset of maternal EV disease in the 2 weeks before delivery. Often, the clinical presentation in neonates mimics that of fulminant bacterial sepsis, so a viral etiology is not considered. When death occurs due to EV infection, it is typically due to hepatic failure with coagulopathy and/or myocarditis.

Severe infections with HPeV (predominantly HPeV3) such as sepsis-like illness and CNS infections have been described in infants less than 3 months of age (23, 24, 96–99). Meningitis or encephalitis due to HPeV infection are often associated with a normal CSF cell count and normal to high protein level (100). White-matter injury can be visualized with cranial ultrasonography or magnetic-resonance imaging (MRI) in the form of increased echogenicity in the periventricular white matter of neonates with HPeV encephalitis (101). Clinical characteristics of HPeV3 sepsis-like illness in 15 neonates and young infants found that 80% developed...
erythematous palmar-plantar rash within 5 days of the onset of fever (102). Because of the potential for substantial morbidity and mortality in these patients, it is important to perform viral diagnostic testing for both EV and HPeV, preferably early in the course of illness.

Most survivors of severe neonatal EV disease do not have long-term cardiac or hepatic dysfunction. However, there are rare reports of residual myocardial dysfunction, chronic calcific myocarditis with chronic heart failure and dysrhythmias, and ventricular aneurysm (103, 104). Some studies suggest that those with EV or HPeV CNS involvement may have long-term sequelae, such as delayed speech and language development, intellectual deficits, motor abnormalities, seizure disorders, ocular defects, or microcephaly (101).

Neonates are also at increased risk of nosocomial EV and HPeV infections, as evidenced by reports of epidemic spread and sporadic transmission occurring in hospital nurseries (105–112). The attack rate for infants at risk has been estimated to range from 22% to 53% in this setting. Generally, nosocomial EV and HPeV infections are associated with lower rates of morbidity and mortality than those of vertically acquired infections (27). The most common risk factors for nosocomial acquisition of EV infection among neonates in the nursery include prematurity, low birth weight, intensive care, and the use of nasopharyngeal or oropharyngeal instrumentation (113).

**HIV/AIDS**

Human immunodeficiency virus (HIV)-associated immune-system dysfunction is characterized by a slowly progressive and complex impairment of immune functions, in particular antigen presentation and functions mediated by CD4+ T lymphocytes. The main effect is an impairment of cellular immunity, although at some stages of disease, humoral immunity can subside. Severe EV disease is observed only sporadically in patients with only cell-mediated immune defects, as illustrated by the absence of reported opportunistic EV infections in acquired-immunodeficiency syndrome (AIDS) patients. No studies to date have described the incidence or severity of HPeV infections in HIV patients.

In the course of HIV infection, symptomatic respiratory-tract infections due to EV are uncommon; only as many as 3% of patients present with signs and symptoms of respiratory-tract illness (60, 114–116). While gastrointestinal disorders frequently affect HIV-positive patients, prospective studies on excretion of EV have found little or no shedding and no significant association with gastrointestinal illness (117–120). No causal link for EVs in HIV-associated cardiomyopathy has been found (121). A variety of neurological syndromes of uncertain etiology can occur during the course of HIV infection, but there are scant reports of CNS infection caused by EV (122). VAPP with Sabin poliovirus type 2 has been reported for one HIV-positive child in Africa and one in Romania (123, 124). A protracted outbreak of EV71 infections has been described among eight HIV-infected children in Kenya in 1999, during which time large EV71 pandemics were occurring in Southeast Asia and Australia (125). Clinical findings included myalgias, excoriation and lip swelling, and dermatitis. No life-threatening complications were noted.

Sporadic asymptomatic shedding of EV during the course of HIV infection has been described for up to 28% of individuals (117–120). Prolonged excretion of vaccine-associated polioviruses is uncommon, but can be significantly higher in HIV-infected children after 3 or more doses of OPV compared to HIV-uninfected children (126, 127). In a study correlating HIV infection and neutralizing antibodies against poliovirus serotypes 1, 2, and 3 after OPV administration, compared to HIV-uninfected children, those with HIV had significantly lower rates of seroconversion to all three types of poliovirus (128). Notably, none of the HIV-infected children were receiving antiretroviral therapy.

**Vaccine-Associated Paralytic Poliomyelitis**

Persons with immunodeficiency, either hereditary or acquired, are at risk for infection with live, attenuated vaccine-derived polioviruses (VDPVs) contained in the OPV. VDPVs can be characterized on the basis of epidemiologic factors: strains associated with immune deficiency (iVDPV), strains associated with person-to-person or community transmission (cVDPV), and “ambiguous” strains, for which the epidemiology is unknown (aVDPV). The primary identified underlying conditions have included B-cell-associated immunodeficiencies (e.g., XLA, common-variable immunodeficiency, transient hypogammaglobulinemia of infancy, IgG subclass deficiency) or T- and B-cell immunodeficiency (e.g., SCID). Individuals with these conditions also represent a potential reservoir for reintroduction of polioviruses because of chronic excretion of vaccine strains. After administration of OPV to immunocompetent persons, the vaccine viruses are excreted for a limited period of time, typically less than 2 to 3 months. Chronic, prolonged shedding of VDPVs is relatively uncommon in patients with primary immunodeficiencies, particularly if they are receiving regular IVIG therapy (129–132). However, some immunodeficient
individuals may excrete vaccine strains for 10 years or longer (133, 134).

Although OPV is not recommended for immunodeficient patients, it is often administered inadvertently because certain primary immunodeficiencies are not recognized until later in life. Selective pressures in the human intestine can cause partial reversion of the attenuated OPV strains to neurovirulence, resulting in VAPP, which is clinically indistinguishable from the paralysis caused by wild-type virus, affecting both vaccine recipients and their contacts. The risk of VAPP is highest after the first dose of OPV, estimated as 1 case per 750,000 children vaccinated, and it is 3,000 times higher for immunocompromised persons. Some patients with B-cell-associated immunodeficiency who excrete VDPVs may experience onset of poliomyelitis several years after the implicated OPV dose was administered (133, 135). Some patients who received immunoglobulin-replacement therapy and excreted virus for prolonged periods of time spontaneously stopped shedding VDPVs (136–138). Four patients with SCID and persistent, nonparalytic, vaccine-related poliovirus infection ceased shedding polioviruses after successful bone-marrow transplantation with reconstitution of humoral immunity, as evidenced by increased levels of serum-neutralizing antibodies to the respective poliovirus serotypes (139).

**TREATMENT**

Since the primary mechanism of clearance of EV by the host is humoral immunity, those patients with impaired antibody production because of congenital or acquired immunodeficiency are particularly susceptible to infection. Similarly, neonates are at high risk for severe enteroviral and parechoviral disease because of a relative deficiency of antibodies. Immunoglobulin preparations have been used prophylactically and therapeutically against EV and HPeV disease. In neonates with disseminated disease, some clinical success has been achieved with the use of maternal serum or plasma or commercially prepared immunoglobulin preparations against a number of EV serotypes (26, 109, 140). However, some infants may have progressive disease and die despite such therapy (141, 142). IVIG has been used as supportive therapy in a case of severe HPeV infection, but randomized studies are needed to prove therapeutic efficacy (143). Early IVIG therapy may be beneficial for survival in neonates with severe EV infections (144).

With the early recognition of immunodeficiency and treatment with IVIG, the incidence and severity of chronic, progressive EV meningoencephalitis in patients with congenital or acquired antibody deficiencies has declined (145). However, results of IVIG therapy in those patients with established CNS infection have been mixed. Despite ongoing IVIG therapy, some patients may have evidence of viral persistence in the CNS, as demonstrated by RT-PCR and progressive neurologic deterioration (33, 41). The failure of IVIG therapy in these patients may be related to the low concentrations of antibodies achieved in CSF (146). Additionally, IVIG only replaces IgG and is unable to correct the secretory immune defect. Moreover, the use of nonselected lots of IVIG does not provide uniformly high levels of serotype-specific antibody (147). There is some evidence that the disease can be stabilized by infusing immunoglobulin-containing antibodies to the relevant EV serotype into the CSF, usually through an Ommaya ventricular reservoir (148).

Currently there is no specific antiviral therapy available for EV or HPeV infections. The most studied antiviral compound is pleconaril (Schering-Plough, Kenilworth, NJ), which has been used in trials of healthy hosts with common EV infections as well as antibody-deficient individuals with potentially life-threatening infections. Pleconaril integrates into the capsid-canyon hydrophobic pocket, preventing viral replication by inhibiting viral uncoating and blocking viral attachment to host-cell receptors. Although it has been evaluated in a number of patient populations, pleconaril is not currently available for treatment. Several other candidate capsid-inhibitor compounds with EV activity are said to be in clinical development (149).

A small series of patients with humoral immune deficits who received pleconaril included 17 patients with CEMA, 6 neonates with disseminated EV infection, 2 patients with severe EV infection following bone-marrow transplantation, and 2 patients with VAPP (46). Twelve of the patients with CEMA showed clinical improvement after pleconaril treatment, and viral clearance in the CSF was noted for six of seven patients who had subsequent CSF samples taken. Five newborns with overwhelming infection survived with only mild sequelae, and both BMT recipients had clinical improvement. Other reports have shown similar efficacies of pleconaril in newborns with fulminant infection and patients with CEMA (150–152); however, in a few patients who failed to respond, the infecting EV strain was formally shown to be resistant (153). Moreover, a survey of 215 clinical EV isolates has shown that about 10% may be resistant to the levels of pleconaril likely to be achieved in vivo (i.e., about 2 μg/mL) (154). Treatment of a chronic enteral HPeV infection with pleconaril
in a patient with common-variable immunodeficiency had no effect on virus clearance (155).

LABORATORY DIAGNOSTIC TECHNIQUES
A specific diagnosis of EV or HPeV infection requires detection of the virus itself, of EV- or HPeV-specific antibodies, or of the viral genome in patient samples. Because of the myriad clinical manifestations of both EV and HPeV infections, confirmation of the diagnosis can be important in reducing hospitalization, antibiotic use, and additional diagnostic testing often performed to exclude or treat other conditions (156). Available methods to identify EV and HPeV infections include nucleic-acid detection, culture, serology, and antigen detection.

Nucleic-Acid Detection
Nucleic-acid amplification methods, such as RT-PCR and nucleic-acid sequence-based amplification, have replaced culture-based techniques as the gold standard for detection of EV and HPeV, given the significant increase in sensitivity over other methods and the rapid turnaround time. This is particularly notable for testing of CSF specimens for the diagnosis of aseptic meningitis, where isolation of EV by conventional cell culture requires mean incubation times of 3.7 to 8.2 days and is up to 75% less sensitive than RT-PCR (157–161). The rapid diagnosis (as few as 3 to 5 hours) of EV meningitis afforded by RT-PCR allows for significant healthcare-associated cost savings, such as decreased length of hospitalization and antibiotic or antiviral usage (8, 156, 160, 162). For some patients, CSF may be positive for EV by culture or RT-PCR despite normal cell counts and chemistries. RT-PCR is also more sensitive than culture for identification of EV in respiratory-tract secretions, urine, and blood (8, 161, 163). It has been used to detect enteroviral RNA in cardiac tissue from patients with myocarditis and other heart diseases and in liver tissues from neonates with fulminant infection (164–168).

HPeVs can be detected from stools, respiratory secretions, blood, and CSF (169). RT-PCR of stool has led to the highest detection rate in symptomatic pediatric patients and fecal HPeV shedding can occur up to two months after the onset of symptoms. HPeVs have been detected in blood during the acute phase of infection in children with severe disease and in the CSF of patients <3 months old with sepsis-like syndrome or signs of meningitis (23, 143). For neonates and young infants, testing of blood for EV or HPeV is often a more sensitive indicator of active infection than testing by RT-PCR with CSF specimens (163, 170, 171). Testing of blood can identify EV and HPeV in a substantial proportion of sepsis cases, some of which will be missed if only CSF is tested. In one study, serum RT-PCR was the only positive test for nearly one-quarter of infants <90 days of age with symptoms of sepsis (2). Combined testing of CSF and serum for infants and young children can increase the diagnostic yield for those with sepsis and/or CNS disease by up to 20% (7, 172, 173). For infants and children with EV infection, testing of urine or throat-swab specimens by RT-PCR is up to 50% more sensitive than culture but less likely to be positive than testing by RT-PCR with CSF and/or blood (8, 161, 163). For children up to 16 years of age who had multiple specimen types submitted for HPeV RT-PCR, the capability of detection was 95% for stool, 85% for CSF, 79% for blood, 94% for nasopharyngeal specimens, and 57% for urine (169). In pediatric cases of acute myocarditis with or without viral pneumonia, there was a strong correlation between EVs detected in tracheal aspirates and myocardial biopsies (174). In adults with EV meningitis, the yield of CSF testing by RT-PCR more than 2 days after the onset of clinical symptoms is often less than that of culture or RT-PCR of stool specimens (175). However, in both adults and children, the finding of asymptomatic colonization and shedding of EV or HPeV in sites such as the respiratory and gastrointestinal tract precludes strictly defining the virus as the etiology of disease when identified only in these types of specimens. In other words, the positive-predictive value of infection based only on identification of EV or HPeV in the oropharynx or stool is significantly less than that of detection in nonpermissive sites, such as blood and CSF.

Most EV molecular assays have been designed to detect genomic sequences within the 5′-nontranslated region, where short spans of nucleotides with high sequence identity exist among the majority of serotypes. Notably, some EV serotypes may not be detected by all RT-PCR assays that use this target region and HPeVs are not detected with EV-specific molecular assays; different primers targeting the 5′-nontranslated region need to be used for detecting HPeV (176). Some investigators have reported using laboratory-developed assays that can simultaneously detect EV and HPeV in clinical samples by using multiplex one- or two-step real-time RT-PCR (173, 177–179). Specific typing of EV and HPeV can be accomplished by sequence analysis of the highly variable VP1 region (176). Although cross-amplification in EV RT-PCR assays with bacteria, fungi, and most nonrelated viruses has not been seen, high sequence identity with rhinoviruses within the 5′-nontranslated region has resulted in the amplification of certain sero-
types, depending on the primers and/or probe sequences utilized (180–182). This cross-reactivity is notable for testing of respiratory samples, but not for CSF, blood, or urine, since rhinoviruses have not been isolated from the CNS and only occasionally from other compartments in cases of overwhelming infection.

Several FDA-cleared molecular assays are currently available for detection of enterovirus (along with rhinovirus) in respiratory samples. These include the FilmArray Respiratory Panel (bioMérieux), the xTAG Respiratory Viral Panel (Luminex), and one real-time RT-PCR assay specific for detection of EV-D68 that was designed and developed by the Centers for Disease Control and Prevention (CDC). Both FilmArray and xTAG assays show cross-reactivity between enteroviruses and human rhinoviruses (HRVs) and are reported as a single result that does not distinguish between the two. The FilmArray Respiratory Panel is a multiplex PCR system that can detect 20 respiratory viruses and bacteria in approximately one hour. The assay uses six sets of broadly reactive primers that amplify RNA from either HRV or EV and results are reported as “human rhinovirus/enterovirus”. The xTAG is a suspension-array detection system following multiplex PCR and the assay can detect up to 12 different respiratory virus targets simultaneously including a combined result for HRV/EV. In head-to-head evaluations of the FilmArray and xTAG, the sensitivity of detection of HRV/EV ranged from 84% to 96% and 91% to 98%, respectively with specificities for both assays ranging from 98% to 100% (183–185). The CDC EV-D68-specific assay has demonstrated sensitivities of 98% to 100% and specificities of 92% to 98% for testing of respiratory specimens (186, 187).

Currently, two FDA-approved real-time assays are available for the detection of EV from CSF only. There are no FDA-approved molecular assays for HPeV detection at this time, although a number of pan- and type-specific laboratory developed assays have been described (176–179, 188, 189) (Table 3). The Xpert EV assay (Cepheid, Sunnyvale, CA) is performed on the Cepheid GeneXpert System. This is a self-contained, random-access, fully automated system in which sample preparation, amplification, and real-time multiplex detection are integrated in a single module for detection of EVs in approximately 2.5 hours (190–192). The sensitivity of the Xpert assay for detection of EV in CSF samples ranges from 94% to 100%, with a specificity of 100%, a positive-predictive value of 100%, and a negative-predictive value of 98% to 99% (190–192). The NucliSENS EasyQ Enterovirus assay (bioMérieux, Durham, NC) is a nucleic-acid sequence-based amplification (NASBA) test that requires a separate nucleic-acid extraction prior to testing on the NucliSENS EasyQ platform using real-time molecular-beacon detection (180). The sensitivity of the NucliSENS EasyQ assay for detection of EV in CSF samples ranges from 88% to 97%, with a specificity of 100% (180, 191, 193, 194). In May 2015 the FDA issued an emergency-use authorization (EUA) to authorize the use of the CDC EV-D68 qualitative RT-PCR assay for testing of upper respiratory-tract specimens and sera from patients with signs and symptoms compatible with EV-D68 infection. Although the expected diagnostic yield of serum testing is low, viremia has been detected in a small number of individuals. The performance characteristics of selected commercially available and laboratory-developed

### Table 3 Performance characteristics of selected molecular assays for detection of EV and HPeV RNA

<table>
<thead>
<tr>
<th>Assay (manufacturer)</th>
<th>Regulatory status</th>
<th>Specimen types</th>
<th>Sensitivity (%)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xpert EV (Cepheid)</td>
<td>IVD</td>
<td>CSF&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94 – 100</td>
<td>190–192</td>
</tr>
<tr>
<td>NucliSENS EasyQ Enterovirus (bioMérieux)</td>
<td>IVD</td>
<td>CSF&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88 – 97</td>
<td>180, 191, 193, 194</td>
</tr>
<tr>
<td>CDC EV-D68 RT-PCR</td>
<td>IVD</td>
<td>Respiratory/blood</td>
<td>98 – 100</td>
<td>186, 187</td>
</tr>
<tr>
<td>FilmArray Respiratory Panel (EV/HRV&lt;sup&gt;α&lt;/sup&gt;</td>
<td>IVD</td>
<td>Respiratory</td>
<td>84 – 96</td>
<td>183–185</td>
</tr>
<tr>
<td>(&lt;bioFire&gt;)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>xTAG Respiratory Viral Panel (EV/HRV&lt;sup&gt;α&lt;/sup&gt;</td>
<td>IVD</td>
<td>Respiratory</td>
<td>91 – 98</td>
<td>183–185</td>
</tr>
<tr>
<td>(Luminex)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-gene Enterovirus and Parechoivirus (bioMérieux)</td>
<td>RUO&lt;sup&gt;b&lt;/sup&gt;</td>
<td>CSF&lt;sup&gt;c&lt;/sup&gt;</td>
<td>94 (EV)</td>
<td>243–245</td>
</tr>
<tr>
<td>Noncommercial EV and/or HPeV reagents</td>
<td>LDA&lt;sup&gt;d&lt;/sup&gt;</td>
<td>CSF/blood/stool respiratory/urine</td>
<td>89 – 100</td>
<td>170, 176–179, 188, 189, 246–250</td>
</tr>
</tbody>
</table>

<sup>a</sup>IVD, in vitro diagnostic (FDA-approved).  
<sup>α</sup>HRV, human rhinovirus.  
<sup>b</sup>RUO, research use only.  
<sup>c</sup>LDA, laboratory-developed assay.  
<sup>d</sup>CSF, cerebrospinal fluid; RT-PCR, reverse-transcription polymerase-chain reaction.
real-time PCR assays for detection of EV and/or HPeV are listed in Table 3.

The correlation between EV disease severity and blood viral load has been assessed using a laboratory-developed real-time RT-PCR on serial samples from infants of <2 months of age (195). For all patients, the highest viral-RNA load was detected in the initial blood specimen, with a subsequent gradual decrease over time. Those patients with severe, disseminated disease had higher initial viral loads (>23,000 copies/μl) and viremia that persisted for up to 2 months, while those with mild disease had initial viral loads <2,000 copies/μl, with viremia persisting no longer than 10 days. A rapid and significant decrease in viral load was seen for two patients with severe disease following administration of IVIG. In another study evaluating patients with EV meningitis, viral loads in CSF from neonates were found to be higher than those in young children, but were not different from viral loads in adults (196). In neonates, viral loads were higher in the presence of pleocytosis than in the absence of pleocytosis. Currently, there are no FDA-cleared or approved quantitative EV molecular assays and no universally accepted quantitative standards. This precludes the use of viral-load values routinely in clinical practice and the aforementioned cutoff values may not necessarily be applicable in settings with different methodologies.

Molecular methods generally have a high sensitivity to detect EV RNA in formalin-fixed, paraffin-embedded (FFPE) tissue, although there are some limitations. A low viral load in the specimen or poor-quality fragmented RNA due to formalin fixation can result in false-negative results. The large number and diversity of EV serotypes also makes it difficult to detect them all using a single oligonucleotide probe for in situ hybridization (ISH). Both positive- and negative-sense RNA can be detected using ISH, primarily in patients with heart disease (166, 197). It has also been described for use with muscle-biopsy specimens and placental material (93, 198). EV RT-PCR on FFPE tissue is frequently positive in cases that are negative by ISH or immunohistochemistry (IHC), but show histopathologic features of EV infection.

Culture
Isolation of EV in cell culture has historically been the mainstay for diagnosis, particularly since most strains grow readily. However, no single cell line allows for propagation of all EV serotypes. In addition, some EV serotypes fail to grow in cell culture. This is particularly true for a number of the group A coxsackieviruses, for which isolation by inoculation of suckling mice has historically been used. Viral cultures have been used for the diagnosis of HPeV infections, but propagation has proven to be difficult. In comparison with HPeV1 and HPeV2, HPeV3 grows less efficiently and only in a limited number of cell lines (199). Most laboratories do not routinely use the Vero (African green monkey kidney) cell line that provides optimal growth. Additionally, typing reagents for HPeVs isolated in cell culture are not broadly available.

In the clinical laboratory, the principal cell lines used for recovery of EV include primary monkey kidney, A549 (human lung carcinoma), buffalo green monkey kidney (BGMK), human embryonic lung fibroblast, and RD cells, which are derived from a human rhabdomyosarcoma. Polioviruses, group B coxsackieviruses, and echoviruses grow efficiently in monkey kidney cells, and many group A coxsackieviruses can be isolated using human embryonic lung fibroblast and RD cells (15, 200). Typically, detection of cytopathic effect (CPE) in these cell lines requires five or more days of incubation. HPeV types 1 to 6 replicate in RD, A549, and Vero cells, and all but HPeV3 show efficient growth on HT29 (human colon carcinoma) cells (199). Thus, a combination of HT29 and Vero cells would support replication of all cultivable HPeV types. CPE may not be observed for all strains, so HPeV-specific mAbs or RT-PCR may be needed to specifically identify HPeV in cell culture.

The sensitivity of cell culture is highly dependent on adequate collection, handling, and processing of specimens. Recovery of virus may be affected for these reasons as well as by antibody neutralization in situ or because of the intrinsic insensitivity of the cell lines used. Recovery of virus in cell culture is optimized by sampling multiple sites. For example, viral-culture specimens with the highest yields in neonatal EV infections are rectum or stool (88% to 93% positive), CSF (62% to 83% positive), and nasopharynx or throat (52% to 67% positive) specimens (26, 84, 201). Cultures of serum or urine have lower yields (24% to 74%) than do cultures from mucosal sites; however, serum specimens may grow virus more rapidly than do specimens from other body fluids/sites. Cultures of serum are more likely to be positive with echoviruses, low serum neutralizing-antibody titers, and an onset of illness within the first 5 days of life. In infants and young children with EV infection, most can be found to be shedding virus by stool culture (70% to 80%), whereas only 44% to 66% of CSF, 48% to 68% of throat or nasopharynx, 42% to 58% of urine, and 37% to 39% of blood samples will yield an EV in cell culture (8, 161, 171, 201). For adults with aseptic meningitis, culture of stool specimens typically has a
high yield (175). For immunocompromised patients with CNS infection receiving IVIG, the rates of recovery of EV from CSF by culture are quite low compared to those for RT-PCR (42).

Centrifugation-enhanced inoculation and immunofluorescence or immunoperoxidase staining prior to development of CPE in shell-vial cell cultures have been effective at decreasing the time to detection of EV from various sources (202–207). By this method, EVs that may replicate poorly are also detected. Fifty-seven percent to 94% of EV-containing cultures are positive by this method within 72 hours postinoculation, whereas only 23% to 51% of tube cultures are CPE-positive in the same time frame (203–205, 207). However, because these centrifugation-enhanced cultures are routinely stained blindly only for a specific virus or viruses at a designated time interval rather than evaluated for CPE, only the viruses sought will be detected, and unanticipated viruses will be missed. In fact, for persons with immunosuppression, there is heightened potential for coinfection with more than one virus in respiratory specimens. Although the frequency of mixed infections is difficult to estimate, it has been shown that up to 26% of respiratory samples from immunocompromised patients contain more than one virus (208).

Transgenic and cocultured cell lines can increase the sensitivity of recovery of EV and eliminate the need for multiple shell vials. BGMK cells stably transfected with human decay-accelerating factor, a cofactor involved in the binding and entry of some echoviruses, coxsackieviruses, and EV70, have been cocultured with CaCo-2 cells, a human colon-adenocarcinoma cell line, or A549 cells (209) (Super E-Mix; Diagnostic Hybrids, Athens, OH). These mixed cell cultures have been shown to be more sensitive for recovery of EV from clinical samples than conventional tube or shell-vial cultures with single cell lines (158, 209).

Typing of EV and HPeV is useful for continued public-health surveillance and, in certain circumstances, for clinical diagnostic purposes, since there are differences noted in the predominant clinical manifestations and outcomes associated with different EV and HPeV types and various sensitivities to antipicornavirus medications currently under development. In addition, in those areas where use of OPV occurs, it is important to distinguish between isolates of the vaccine-strain polioviruses and the nonpoliovirus EVs. Traditionally, EV serotypes have been determined by using intersecting pools of lyophilized antisera adopted by Lim and Benyesh-Melnick (LBM pools) (210). Alternatives to the LBM pools include serotype-specific MAb and pan-reactive antibody preparations, some of which are commercially available for cell-culture confirmation by immunofluorescence (Diagnostic Hybrids, Athens, OH; EMD Millipore, Billerica, MA). Cross-reactions of pan-reactive reagents with other viruses, such as hepatitis A, reovirus, astrovirus, adenovirus, and rhinovirus, have been demonstrated (203). More recently, molecular-typing methods have been developed to identify individual EV and HPeV types. These include VP1-gene sequence-based typing and type-specific PCR assays (177, 211–213).

**Serology**

Serology has limited clinical value in routine diagnosis of EV or HPeV infections because of the time needed to obtain acute- and convalescent-phase samples, the large number of serotypes that exist, and the lack of a common antigen for use in serologic assays. Documenting a rise in serotype-specific IgG antibody titer of ≥4-fold from the acute to the convalescent phase of infection may be useful in epidemiologic investigations, such as studies of outbreaks of a specific EV or HPeV serotype. Serotype-specific antibodies can be determined using neutralization, complement fixation, or hemagglutination inhibition. Generally, these assays have limited usefulness because they are relatively insensitive, poorly standardized, and labor-intensive and they lack specificity (214–216). Enzyme immunoassays (EIAs) for group- and serotype-specific determination of IgA, IgM, and IgG are also often limited by their sensitivity and specificity (215–218). For culture-confirmed cases of EV infection, the sensitivities and specificities of IgM detection by EIA range from 34% to 77% and 88% to 94%, respectively (215, 216, 219).

**Antigen Detection**

IHC detection of EV has been described primarily for the diagnosis of myocardial infections but has also been used for other tissues, such as the spleen, lung, kidney, intestine, bone marrow, and pancreas (62, 166, 167, 220, 221). Localization of the virus in tissue can be of importance for understanding viral pathogenesis. IHC for detection of EVs is typically more sensitive than ISH techniques, likely due to RNA loss or degradation during fixation or histological processing (197). However, compared to RT-PCR of tissue sections, reported sensitivities of IHC range from 57% to 100% (164, 166, 167). Although the manufacturer’s indications for use of anti-EV antibodies are limited to culture confirmation and identification, fluorescent-antibody techniques using serotype-specific mAbs on tissue sections have
been reported (168). Detection of EV antigen directly in stool samples by EIA has been reported to be as sensitive as traditional cell culture, but only 58% sensitive compared to RT-PCR (222).

**Antiviral-Susceptibility Testing**

While not routinely used in clinical practice, in vitro susceptibility testing of pleconaril against a number of EV serotypes, using a well-characterized cell-culture protection assay, has been reported (154). Serial dilutions of drug were added to 96-well cell-culture plates infected with the EV serotype of interest, and after 3 days, the monolayers were examined for CPE. The concentration of drug that protected 50% of the cell monolayer from virus-induced CPE was defined as the 50%-inhibitory concentration. Of 215 clinical isolates representing the 15 most common EV serotypes and 15 prototypic EV strains, all but 2 were inhibited by pleconaril, with 50%-inhibitory concentrations of ≤3.4 μM. One prototypic strain of coxsackievirus B3 and one clinical isolate of echovirus 24 were insensitive to the drug, likely due to amino-acid changes in the drug-binding pocket within the capsid (154, 223).

The antiviral susceptibility of EV71 to pleconaril has been tested using both an in vitro and in vivo model (224). The viability of EV71-infected RD cells treated with serial dilutions of drug could be assessed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay. In a model using one-day-old mice infected with a fixed concentration of EV71 and subsequently treated with varying doses of pleconaril, a clear dose-dependent increase in survival and reduction in disease severity was demonstrated. Such tools could potentially be used to evaluate the antiviral activity of new compounds against various EV and HPeV isolates.

**Test Selection, Application, and Interpretation**

EV and HPeV can be detected in multiple specimen types, such as blood, CSF, urine, or tissues, depending on the clinical syndrome (Table 4). Identification of EV or HPeV in these sites is associated with a high likelihood of causality for the disease state. For example, detection of HPeV in the CSF of a newborn with encephalitis should be considered diagnostic, whereas detection in the stool or nasopharynx of an older child may reflect coinfection, with symptoms likely attributable to another agent. Some individuals may be asymptomatically colonized and shed EV or HPeV in the oropharynx or stool for weeks to months. OPV strains are also shed in stool for weeks after administration and will be detected in cell culture.

If EV or HPeV infection is suspected in an immunocompromised patient, employment of a rapid diagnostic method is desirable, not only so treatment can be instituted as early as possible, but also to reduce unnecessary diagnostic evaluations and treatments. Nucleic acid-amplification assays are appropriate for CSF, serum, plasma, stool, urine, respiratory, and tissue specimens. Cell culture for EV is appropriate for CSF, stool, respiratory, and tissue specimens, and ISH/IHC is appropriate for tissue specimens. However, while cell culture allows for identification of a broad range of viruses, the time required for isolation and identification may be too long to be of clinical utility. Nucleic acid-amplification assays typically afford greater sensitivity along with more rapid time to result. The specificities of nucleic acid-amplification assays are generally excellent. The notable exception to this is testing of respiratory specimens, where rhinoviruses may also be present. It behooves the testing laboratory to determine the validity of their EV assay for respiratory samples by assessing potential

**TABLE 4** Suitability of specimen types for diagnosis of EV or HPeV infections.<sup>a,b</sup>

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>CSF sens/spec</th>
<th>Blood sens/spec</th>
<th>Respiratory sens/spec</th>
<th>Urine sens/spec</th>
<th>Stool sens/spec</th>
<th>Tissue sens/spec</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS infection</td>
<td>EV RT-PCR +++/+ +++/+++ +++/+ +++/+ -/-</td>
<td>++/+ ++/+ ++/+ ++/+ -/-</td>
<td>++/+ ++/+ ++/+ ++/+ -/-</td>
<td>++/+ ++/+ ++/+ ++/+ -/-</td>
<td>++/+ ++/+ ++/+ ++/+ -/-</td>
<td></td>
</tr>
</tbody>
</table>
cross-amplification with many of the more common rhinovirus serotypes.

RT-PCR is particularly useful for documenting EV infection in those patients with B-cell-associated immunodeficiencies, where recovery of viruses in cell culture is inadequate, particularly if IVIG has been administered. During the course of CEMA, RT-PCR testing of CSF may intermittently be negative, but this result should not exclude the diagnosis, and testing of subsequent CSF samples should be attempted. For those patients with immunodeficiency and EV infection, the sequential recovery of virus over time has provided an opportunity to study the genomic changes that EV strains undergo during prolonged replication in a human host (225–227). Viral-load testing by real-time RT-PCR may prove to be a useful tool for some patient populations (e.g., neonates) for early diagnosis and prediction of disease severity, although assays for this type of testing are not yet commercially available.

For neonates, sampling from multiple sites increases the diagnostic yield of EV and HPeV. The identification of EV or HPeV from a mucosal site in a neonate along with virus-compatible illness and the absence of other infectious or noninfectious causes are strong indications that EV or HPeV is the likely etiologic agent.

Interpretation of serologic results with paired serum samples may be complicated by the high prevalence of EV and HPeV antibodies in the general population and the fact that antibody may already be present in the acute sample due to long incubation and prodromal periods. Interpretation of EIA results is complicated by the heterotypic responses of many individuals caused by other EV infections and therefore cannot be considered strictly serotype-specific (215, 219). The performance characteristics of the serologic assays and the fact that a large percentage of immunocompromised individuals will have diminished antibody production preclude the routine use of this type of testing for diagnostic purposes.

EV vaccines are available for the three poliovirus serotypes, but they provide no protection against the nonpoliovirus EV serotypes. Live viral vaccines such as OPV are contraindicated for immunocompromised patients (228). Only IPV should be administered. In addition, it is recommended that family members, close contacts, and healthcare workers of immunodeficient individuals be given only the IPV (229). With regard to nonpoliovirus EV, formalin-inactivated EV71 vaccines have been evaluated in human clinical trials in Asian countries and were found to be safe and to elicit strong neutralizing-antibody responses against currently circulating EV71 strains. From phase three clinical trials performed in young children, the efficacy of EV71 vaccines is >90% against EV71-related HFMD and >80% against EV71-associated serious disease (230).

Because stem-cell-transplant recipients lose immunity to poliovirus early after transplantation, a revaccination schedule with three doses of the IPV is recommended (229, 231). Revaccination after stem-cell transplantation results in protective immunity in most patients, although a few patients may become seronegative over the course of several years (232–234). Younger patients and those with graft-versus-host disease are more likely to lose immunity after revaccination. Regular testing of long-term stem-cell-transplant recipients approximately every four to five years for maintenance of antibody levels is recommended post-IPV administration (235).

Ideally, solid-organ-transplant patients should be immunized prior to transplantation, since the response to many vaccines may be diminished in the setting of organ failure. Although there are no data regarding the timing of vaccination after transplantation, it is recommended that vaccination series be started no sooner than 6 to 12 months posttransplantation, since there is an increased risk of graft dysfunction and high-dose immunosuppressive treatment may inhibit an adequate immune response prior to this time (236, 237). Hypogammaglobulinemia has been described for heart-, lung-, and kidney-transplant recipients and has been associated with the development of recurrent infections (238–240). In adult renal-transplant patients evaluated at least 1 year after transplantation, few had protective levels of antibody to all three poliovirus serotypes (241). Four weeks after revaccination with a single booster of IPV, approximately 92% of patients demonstrated protective antibody levels to all three serotypes. Similarly, booster immunization with IPV in children after liver transplantation produced similar serologic responses to those noted in controls (242). Currently, there are no recommendations for routine pre- and postvaccination testing of neutralizing-antibody titers to PV in solid-organ-transplant recipients.

REFERENCES
Enteroviruses and Parechoviruses


Enteroviruses and Parechoviruses


Enteroviruses and Parechoviruses


