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Evaluation of Different Clinical Sample Types in Diagnosis of Human Enterovirus 71-Associated Hand-Foot-and-Mouth Disease▽

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Human enterovirus 71 and coxsackievirus A16 are important causes of hand-foot-and-mouth disease (HFMD). Like other enteroviruses, they can be isolated from a range of sterile and nonsterile sites, but which clinical sample, or combination of samples, is the most useful for laboratory diagnosis of HFMD is not clear. We attempted virus culture for 2,916 samples from 628 of 725 children with HFMD studied over a 3 1/2-year period, which included two large outbreaks. Overall, throat swabs were the single most useful specimen, being positive for any enterovirus for 288 (49%) of 592 patients with a full set of samples. Vesicle swabs were positive for 169 (48%) of 333 patients with vesicles, the yield being greater if two or more vesicles were swabbed. The combination of throat plus vesicle swabs enabled the identification of virus for 224 (67%) of the 333 patients with vesicles; for this patient group, just 27 (8%) extra patients were diagnosed when rectal and ulcer swabs were added. Of 259 patients without vesicles, use of the combination of throat plus rectal swab identified virus for 138 (53%). For 60 patients, virus was isolated from both vesicle and rectal swabs, but for 12 (20%) of these, the isolates differed. Such discordance occurred for just 11 (10%) of 112 patients with virus isolated from vesicle and throat swabs. During large HFMD outbreaks, we suggest collecting swabs from the throat plus one other site: vesicles, if these are present (at least two should be swabbed), or the rectum if there are no vesicles. Vesicle swabs give a high diagnostic yield, with the added advantage of being from a sterile site.

Hand-foot-and-mouth disease (HFMD) is a common febrile illness in young children and is characterized by lesions on the skin and oral mucosa. The skin rash, which may be maculopapular or vesicular, typically occurs on the palms and soles but can also involve the buttocks, elbows, and knees. Mouth ulcers are the most common enanthema, but some patients have herpangina (multiple oral ulcers affecting predominantly the posterior part of the oral cavity), and others have no oral lesions (16, 20).

Many human enteroviruses (family *Picornaviridae*, genus *Enterovirus*) can cause HFMD, but human enterovirus 71 (HEV71) and the closely related coxsackievirus A16 (CVA16) are the most important (16, 20). Since the late 1990s, HEV71 has caused a series of large HFMD epidemics in the Asia-Pacific region, associated with a rapid fulminant course, severe neurological complications, and a large number of fatalities (1–4, 8–11, 14, 17, 18, 21). CVA16 causes a similar clinical illness initially, but neurological and other severe complications are extremely rare (5). In much of Asia, there is now epidemiological and virological surveillance for HFMD so that effective public health measures, such as closing nurseries and schools, can be instituted early. However, because of the similar clinical presentations of the viruses, establishing the actual cause of HFMD cases relies on laboratory identification of the virus. Diagnostic techniques include isolating the virus in susceptible continuous cell lines or detecting viral RNA by PCR (12, 28). Though laborious and time consuming, virus isolation remains the gold standard for enterovirus diagnosis; it is cheaper than PCR and is the most widely used method, particularly in developing countries.

There is a wide range of samples from which virus isolation can be attempted, including rectal and throat swabs, serum, and cerebrospinal fluid (CSF) (when taken) and vesicles and ulcers when they are present. However, for HEV71-associated HFMD outbreaks, there has been relatively little work examining which sample, or combination of samples, is the most useful. This question becomes especially important in the context of large outbreaks with many thousands of patients. Rectal and throat swabs are available for all patients and do not require the presence of mucocutaneous stigmata. However, they have the disadvantage that, because they are not sterile sites, isolation of virus there may represent coincidental asymptomatic carriage rather than the causative agent (24): many enterovirus infections are asymptomatic, and viral shedding may persist for up to 2 weeks from the throat and up to 11 weeks from the rectum (7, 20, 24). In the absence of virus isolation from a sterile site, isolates from nonsterile sites are usually accepted as surrogate markers for enterovirus infec-

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