Laboratory and Epidemiology Communications

Rapid Serological Diagnosis of Enterovirus 71 Infection by IgM ELISA

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Communicated by Tatsuo Miyamura

(Accepted September 30, 2002)

Enterovirus 71 (EV71) is known as one of the major causative agents of hand, foot and mouth disease (HFMD). Furthermore, EV71 infection sometimes is associated with serious central nervous system diseases such as aseptic meningitis, encephalitis, and polio-like paralysis. Recently, large outbreaks of EV71 infection with a number of fatal HFMD cases were reported in the Asia-Pacific region (1). Since some of coxsackieviruses also cause HFMD, the development of a rapid and reliable diagnostic method of EV71 infection is important to differentiate HFMD cases caused by EV71 from those due to other enteroviruses. Virus isolation by cell culture and subsequent identification with the serotype-specific anti-serum have been used as the gold standard of laboratory diagnosis of enterovirus infection. However, these procedures are time-consuming and require specific reagents and equipments. Furthermore, adequate clinical specimens for virus isolation, such as stool and throat swab, are not always collected from HFMD patients by clinicians.

Another approach for reliable diagnosis of acute enterovirus infection is serodiagnostic methods to detect specific IgM antibody in serum samples. A μ-capture enzyme linked immunosorbent assay (ELISA) has been used for various serotypes of enteroviruses, and its sensitivity and specificity have been demonstrated in routine diagnostic use (2-4). However, application of IgM ELISA for rapid serological diagnosis of EV71 infection has not been reported so far. In the present report, we describe the development of an EV71-specific IgM ELISA assay and its preliminary application for clinical specimens from HFMD and other cases.

Twenty serum samples from 11 patients with or without HFMD were applied to the IgM ELISA assay (Table 1). Briefly, 100 μl per well of anti-human IgM monoclonal antibody (ICN/Cappel, Aurora, Ohio, USA), diluted to 1:100 in 0.05 M sodium carbonate buffer, were used to coat the bottom of a 96-well microimmunoplate. The plate was incubated for 1 h at 36°C and washed with washing buffer (0.01 M PBS with 0.05% Tween 20) three times. The serum sample was diluted 1:1000 in dilution buffer (0.01 M PBS with 0.05% Tween 20 and 1% BSA) and 50 μl of each was added to four wells. After incubation for 1 h at 36°C and washing of the plate, native EV71 virus (1095 strain, $10^{5.75}$ $CCID_{50}$), or formalin inactivated EV71/1095 antigen, were added and the plates were incubated for 1 h at 36°C. After washing, anti-EV71 rabbit anti-serum (1:500) was added and incubated for 1 h at 36°C. After washing the plate, horseradish peroxidase conjugated anti-rabbit IgG (1:30000) (ICN/Cappel) was added to each well and the plates were incubated

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Table 1. List of serum samples from cases with or without HFMD

Clinial case	Patient age/gender	Major symptom	Serum	Days after the clinical onset	Neutralization titer against EV71	Virus isolation
1	4y/M	HFMD	A	1	>128	–
			B	8	>128	–
2	7y/M	Encephalitis	C	15	16	–
			D	28	4	–
3	1y/F	HFMD	E	16	>128	–
4	3m/F	HFMD	F	1	>128	+EV71*
			G	8	>128	–
5	3d/F	Myocarditis	H	unknown	64	–
6	Adult/F	Fever	I	unknown	>128	–
7	5y/M	HFMD	J	2	>128	–
8	4y/F	Fever	K	9	<4	–
			L	15	<4	–
			M	22	<4	–
9	1y/M	Encephalitis	N	2	<4	–
			O	6	<4	–
			P	9	<4	–
10	3y/M	Meningitis	Q	2	<4	–
			R	11	<4	–
11	10y/F	Encephalitis	S	0	<4	–
			T	11	<4	–

*EV71 was isolated from throat swab of patient by using RD cell culture.

for 1 h at 36°C. *o*-phenylenediamine substrate with hydrogen peroxide was added and A_{492} was measured. The mean OD value using the diluent instead of the serum sample was used as a negative control.

Most cases tested in this study were clinically suspected to be enterovirus infection. However, virus isolation was not successful due mainly to inadequate sample collection with one exception (Table 1). In fact, the virus isolation rate of EV71 from serum or CSF samples has been reported to be quite low (5). Appreciable rises of neutralizing antibody against EV71 were not identified even for paired serum samples because of high titers even in the first serum sample. On the other hand, anti-EV71 IgM antibody was detected in six out of 20 serum samples (A, B, E, F, G, and J) (Fig. 1). All four HFMD cases were identified to be positive for anti-EV71 IgM antibody. Meanwhile, the other seven patinets without clinical HFMD symptoms were negative when the threshold was set at 0.101 (the mean value of negative control plus 3 SD). Thus, this IgM ELISA system is useful as a simple and rapid serodiagnostic method for EV71 infection especially for a single serum sample. Further investigation using an extended number of clinical samples from cases with or

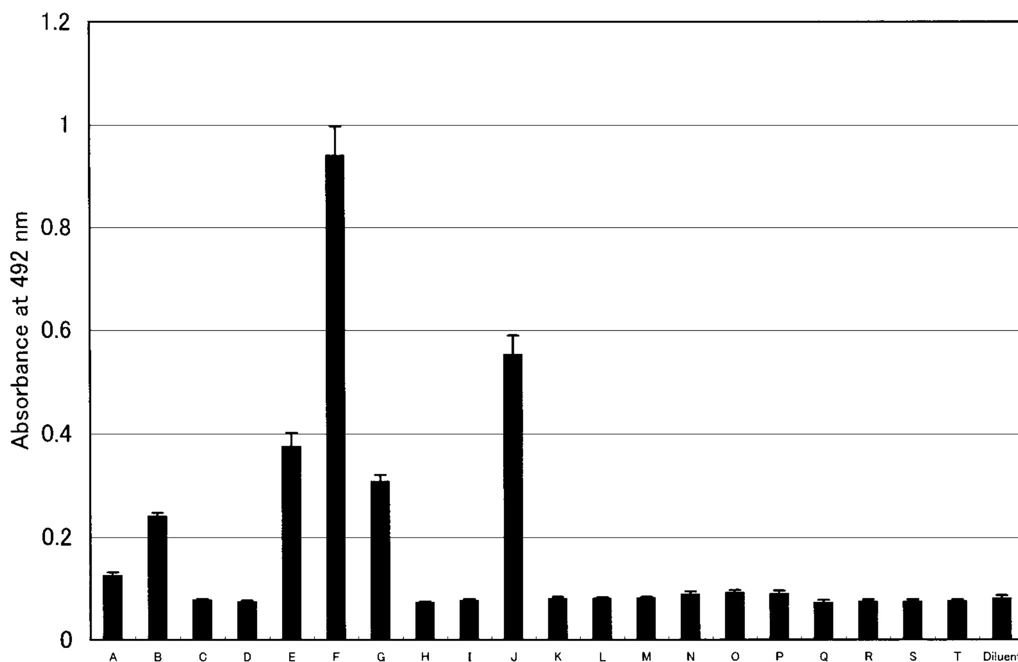

Fig. 1. Detection of anti-EV71 IgM antibody in serum.
The data presents mean OD value of three independent assays plus SD.

without HFMD will be needed to evaluate the reliability of the assay for diagnostic use.

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