Characterization of the antibody response against EV71 capsid proteins in Chinese individuals by NEIBM-ELISA

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(a)

NO.	anti-VP1	anti-VP0	anti-VP3	pET32a
59	1.834	0.255	0.103	0.094
42	1.61	0.28	0.097	0.06
187	1.477	0.282	0.158	0.064
97	1.321	0.295	0.107	0.089
131	1.282	0.278	0.171	0.076
177	1.1	0.289	0.261	0.077
51	1.076	0.255	0.077	0.092
163	0.926	0.287	0.147	0.089
172	0.774	0.279	0.186	0.097
49	0.747	0.264	0.11	0.052
122	0.674	0.287	0.338	0.098
3	0.669	0.272	0.133	0.032
67	0.618	0.304	0.1	0.065
118	0.608	0.301	0.189	0.096
191	0.598	0.281	0.139	0.074
153	0.591	0.282	0.265	0.068
65	0.589	0.269	0.125	0.099
136	0.553	0.286	0.322	0.08
178	0.534	0.296	0.209	0.085
165	0.524	0.3	0.166	0.09
170	0.451	0.278	0.156	0.072
40	0.434	0.263	0.109	0.085
193	0.433	0.269	0.12	0.068
92	0.423	0.278	0.101	0.082
162	0.393	0.286	0.184	0.07
200	0.391	0.288	0.139	0.056
142	0.379	0.279	0.17	0.066
90	0.368	0.302	0.163	0.045
126	0.313	0.294	0.262	0.064
50	0.306	0.264	0.156	0.081
12	0.283	0.276	0.104	0.064
34	0.274	0.281	0.089	0.098
88	0.243	0.28	0.133	0.056
39	0.219	0.278	0.11	0.078
66	0.204	0.28	0.118	0.064

(b)

NO.	anti-VP1	anti-VP0	anti-VP3
67	0.618	0.304	0.1
90	0.368	0.302	0.163
118	0.608	0.301	0.189
165	0.524	0.3	0.166
178	0.534	0.296	0.209
97	1.321	0.295	0.107
126	0.313	0.294	0.262
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142	0.379	0.279	0.17
172	0.774	0.279	0.186
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3	0.669	0.272	0.133
65	0.589	0.269	0.125
193	0.433	0.269	0.12
49	0.747	0.264	0.11
50	0.306	0.264	0.156
40	0.434	0.263	0.109
51	1.076	0.255	0.077
59	1.834	0.255	0.103

(c)

NO.	anti-VP1	anti-VP0	anti-VP3
122	0.674	0.287	0.338
136	0.553	0.286	0.322
153	0.591	0.282	0.265
126	0.313	0.294	0.262
177	1.1	0.289	0.261
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12	0.283	0.276	0.104
59	1.834	0.255	0.103
92	0.423	0.278	0.101
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34	0.274	0.281	0.089
51	1.076	0.255	0.077

Figure S1. Determination of the cutoff values for anti-EV71 ELISA detection in sera samples from normal adults. (a) The samples of anti-EV71 VP1 with OD values below 0.300 (tentative cutoff value) did not react overtly with VP3 or VP0; (b) the samples of anti-EV71 VP0 with OD values below 0.286 (tentative cutoff value) did not react overtly with VP3 and did not always react strongly with VP1; (c) the samples of anti-EV71 VP3 with OD values below 0.200 (tentative cutoff value) did not always react strongly with VP3 and VP1.

Table 1. Baseline characteristics of the study participants

	Blood Donor	non-HFMD Children	Severe HFMD cases
Number[a]	200	194	33
Time	2011.2.24-3.17	2011.6.27-7.4	2011.6.27-7.4
Age (yr)			
Mean ± s.d.[b]	27.0 ± 7.6	3.0 ± 2.4	3.5 ± 2.6
Range	18-48	1-12	1-11
Sex (%)			
Male	52.5%	59.3%	60.6%
Female	47.5%	40.7%	39.4%

[a]Number of evaluable individuals; [b]Standard deviation;

Table 2. Primers for amplifying EV71 capsids and truncated VP1.

Primers	Sequences (5'- 3')	Description
uVP0	GCGCGCGGATCCATGGGTTCTCAGGTTTCT	The primer pairs uVP0/dVP0 and uVP3/dVP3 were used to amplify VP0 and VP3, respectively. uVP0, uVP3 contain BamHI restriction sites (underlined), dVP0, dVP3 contain HindIII restriction site (underlined).
dVP0	GCGCGCAAGCTTTTACTGAGTAACAGCCTGACG	
uVP3	GCGCGCGGATCCGGTTTCCCGACTGAACTG	
dVP3	GCGCGCAAGCTTTTA CTGGATAGTACCAGTCTG	
uVP1-0	GCGCCGGGATCCGGTGACCGTGTTGCTGAC	The primer pairs uVP1-0/dVP1-0, uVP1-1/dVP1-0 and uVP1-2/dVP1-0 were used to amplify $VP1_{1-297}$, $VP1_{41-297}$, $VP1_{61-297}$, respectively. uVP1-0, uVP1-1, uVP1-2 contain BamHI restriction sites (underlined), dVP1-0 contain HindIII restriction site (underlined).
uVP1-1	GCGCGCGGATCCACTGGTAAAGTTCCGGCTCTG	
uVP1-2	GCGCGCGGATCCGAATCTATGATCGAAACTC	
dVP1-0	GCGCCGAAGCTTCTACAGAGTAGTGATAGCAGT	
uVP1-4	CGCGCGGGATCCGGTGACCGTGTTGCTGACGT	The primer pairs uVP1-4/dVP1-4, uVP1-5/dVP1-5,and VP1-6/dVP1-6 were used to amplify $VP1_{1-60}$, $VP1_{134-297}$, and $VP1_{45-58}$, respectively. uVP1-4, uVP1-5, uVP1-6 contain BamHI restriction sites (underlined), dVP1-4, dVP1-5 and dVP1-6 contain HindIII restriction site (underlined).
dVP1-4	GCCGGCAAGCTTTTA GTCAGAAGCGTTAGAAGAAG	
uVP1-5	CGCGGCGGATCCGAATTCACTTTCGTTGCTTG	
dVP1-5	GCCGGCAAGCTTTTACAGAGTAGTGATAGCAGTACGAGA	
uVP1-6	CGCGCGGGATCCCCGGCTCTGCAGGCTGCTG	
dVP1-6	GCCGGCAAGCTTTTA AGCGTTAGAAGAAGCACC	