Validation of solid phase epitope blocking ELISA (SPEBE) to detect antibodies against structural proteins of type O foot-and-mouth disease virus

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Introduction

Sero-surveillance against foot-and-mouth disease virus (FMDV) structural protein antibodies is performed conventionally by solid phase competition ELISA, liquid phase blocking ELISA and virus neutralization test. Although effective and sensitive, these methods require hazardous live virus to be used in the test or for production of the antigen and the LPBE is known to produce a significant number of false positive results. In order to overcome these problems, we developed and used a recombinant P13C (r-P13C) pentamer-like structural protein as antigen and a monoclonal antibody against the RGD motif of O serotype as the competitive detector ligand.

Materials and Methods

The solid phase epitope blocking ELISA (SPEBE) was validated using sera from field outbreaks or vaccinated farms in Vietnam, naïve animals from South Korean farms; international reference sera phase XVIII and sera from experimentally vaccinated and infected animals from vaccine challenge experiments conducted at the Institute for Animal Health, Pirbright, UK.

Results

Testing 2702 sera collected from non-infected cattle, pigs, goats and sheep resulted in a specificity of 99.7%. An equivalent sensitivity to the CEDI® FMDV Type O ELISA (97.4%) was achieved when 352 sera from FMDV-vaccinated animals were tested and 110 of 158 sera from infected animals were positive. All phase XVIII reference sera scored correctly. 100% detection of infection in FMDV O UKG/O Pan Asia (n=199) was observed. However, the detection rate decreased to 96.3% in FMDV O1/Manisa vaccinated animals (n=81).

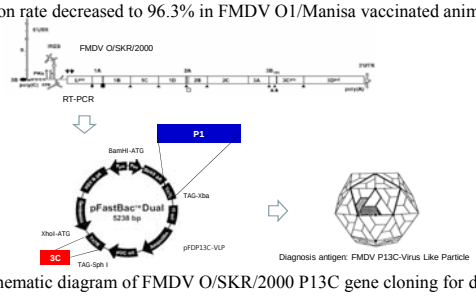

Fig 1. Schematic diagram of FMDV O/SKR/2000 P13C gene cloning for diagnostic antigen.
The transfer plasmids were generated using the pFast-Bac Dual vector (Invitrogen), which contains two multiple cloning sites (MCS) as the backbone. The gene fragments for P1 (2202 bp) and 3C (639 bp) were amplified by PCR from the cDNA of the O/SKR/2000 virus.

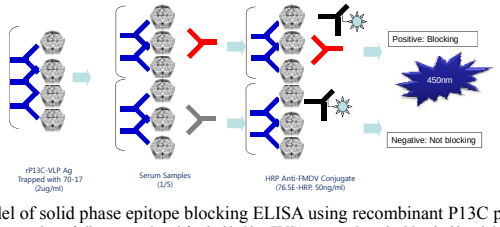

Fig 2.Model of solid phase epitope blocking ELISA using recombinant P13C protein
The optimal concentrations of all reagents adopted for the blocking ELISA were predetermined by checkboard titration. ELISA plate (Greiner, Germany) were coated with purified mAb70.17 (2.0μg/㎖) in 0.05M carbonate /bicarbonate buffer (pH 9.6) at 4 ◦C overnight and the plates were washed with PBS (pH 7.4) that contained 0.05% Tween-20 (PBST). Subsequently, 100 ㎕ of r-P13C antigen was added to each well and incubated with 100 ㎕ aliquots of the test sera (final dilution of 1:5 in PBS-T) at room temperature(RT) for 1h. The plates were washed three times with PBS-T and incubated with 100㎕ of HRP-conjugated mAb76.5E (0.05μg/㎖) at RT for 1h. The optical density at 450 nm of each well was determined in an automated ELISA reader.

Table 1. S/N value distribution of the serum panel (n=538) determined by solid phase epitope blocking ELISA

S/N Value range	No. of samples	Serum panel group		Sensitivity (%)	Specificity (%)
		P[1)]	N[2)]		
0.0	67	67	0	0.0	100.0
0.1	43	41	2	43.5	100.0
0.2	25	25	0	70.1	99.5
0.3	5	5	0	86.4	99.5
0.4	10	10	0	89.6	99.5
0.5	3	2	1	96.1	99.5
0.6	7	1	6	97.4	99.2
0.7	20	2	18	98.1	97.7
0.8	77	0	77	99.4	93.0
0.9	131	0	131	99.4	72.9
>1.0	150	1	149	99.4	38.8
Total	538	154	384		

The sensitivity (97.4%) and specificity (99.2%) of the assay were optimized by setting the S/N ratio threshold value to 0.60.
Virus neutralization titer of positive control serum was more than 64; normal goat serum was used as the negative control
1) P: Virus neutralization titer of positive serum panel group was more than 45
2) N: Virus neutralization titer of negative serum panel group was below 45

Table 2. Result of international reference sera (WRL phase XVIII) exercise by solid phase epitope blocking ELISA, virus neutralization test, the liquid-phase blocking ELISA and the solid-phase blocking

No	Serum	Reference test			SPEBE		
		VNT [1)]	Titer of LPBE [2)]	PI% of SPCE[3)]	OD	S/N	Result
1	Strong positive	237	708	92	0.409	0.26	Positive
2	Weak positive	119	596	85	0.647	0.40	Positive
3	Cut-off	44	178	58	0.927	0.58	Positive

A serum is considered as a positive with SN value < 0.60 for solid phase epitope blocking ELISA(SPEBE); PI% ≥50 for the solid-phase blocking ELISA ; titer ≥ 45 for liquid-phase blocking ELISA and the virus neutralization test.
1) VNT: virus neutralization test; 2): LPBE: liquid-phase blocking ELISA; 3) SPCE: Solid-phase blocking ELISA

Table 3. Sensitivity evaluation in experimentally vaccinated and infected animal sera with FMDV O serotype virus

Group	Species	No. sample	SPEBE		
			Mean S/N	No of positive	Positive rate(%)
O UKG (n=182)	Cattle [1)]	93	0.22	93	100
	Pig[2)]	17	0.15	17	100
	Sheep[2)]	72	0.28	72	100
PanAsia O (n=17)	Cattle[3)]	17	0.13	17	100
Total/Mean		199	0.23	199	100

A serum is considered as a positive with SN value < 0.60 for solid phase epitope blocking ELISA(SPEBE)
1) Cattle was immunized with vaccine from O1 Manisa strain, two times and infected with O UKG strain
2) Pig and sheep was infected with O UKG strain; 3) Cattle was infected with FMDV PanAsia O strain

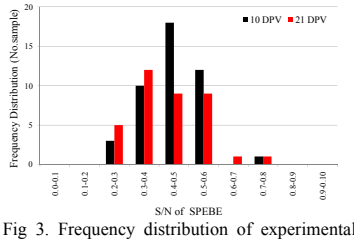

Fig 3. Frequency distribution of experimental sera from FMDV O Manisa vaccinated animals (n=81). The sensitivity was estimated as 96.3% ; DPV: Days of post vaccination

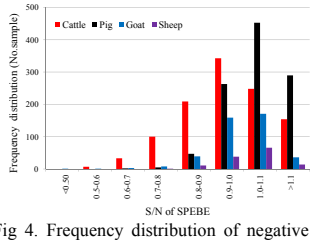

Fig 4. Frequency distribution of negative sera from FMD free areas.
Testing 2702 sera collected from South Korea resulted in a specificity of 99.7%.

Table 4. Comparison result of SPEBE and commercial ELISA with sera from FMD vaccinated areas (n=352)

Species	CEDI® FMDV type O ELISA		SPEBE	
	Result	No. sample	No of positive	No of negative
Buffalo	Positive	36	36	0
	Negative	8	0	8
Cattle	Positive	104	101	3
	Negative	28	1	27
Pig	Positive	11	11	0
	Negative	165	5	160
Total	Positive	151	148	3
	Negative	201	6	195

The samples were collected from 4 province during 2007.11 ~ 2008.3 in Vietnam. Most samples were considerate as vaccinated. In CEDI® FMDV Type O ELISA, 151 samples were positive (42.9%), 154 samples were positive (43.8%) %) in solid phase epitope blocking ELISA(SPEBE). An eq uivalent sensitivity to the CEDI® FMDV Type O ELISA (97.4%) was achieved.

Table 5. Field application in FMDV outbreak farms in Vietnam (n=158)

Species	SPEBE		VDPro® FMDV NSP AB ELISA	
	Result	No. sample	No of positive	No of negative
Buffalo + cattle + pig	Positive	4	3	1
	Negative	1	0	1
Buffalo	Positive	51	22	29
	Negative	26	2	24
Cattle	Positive	55	24	31
	Negative	21	0	21
Total	Positive	110	49	61
	Negative	48	2	46

The tested sera were collected from FMDV outbreak (5 provinces) in 2006. In solid phase epitope blocking ELISA(SPEBE), 110 samples were positive (69.6%). The 2 samples in SPEBE negative were positive in VDPro® FMDV NSP AB ELISA, it was considered as cases of other type of virus infected.

Conclusions

The observed sensitivity and specificity is sufficient to use solid phase epitope blocking ELISA as a screening test for the detection of type O FMDV antibodies. It is possible to replace inactivated FMDV antigen with non-infectious recombinant P13C pentamer-like structures in the ELISAs