

The rapid identification of foot-and-mouth disease virus (FMDV) and its serotype by the indirect immunofluorescent antibody test using monoclonal antibodies reacted with the multi- and single-serotype FMDV

Exotic Diseases Research Station, National Institute of Animal Health, Japan

Katsuhiko Fukai, Kazuki Morioka, Hiroyuki Onozato, Seiich Ohashi
Reiko Yamazoe, Kazuo Yoshida, Kenichi Sakamoto

Object

- Virus isolation has sufficient sensitivity for FMDV detection.
- However, an isolate has to be serotyped by an ELISA or nucleotide sequencing analysis.
- In general, CPE during virus isolation appears approximately 24-72 hours after inoculation and is therefore not a rapid test.



- We attempted to rapidly detect FMDV and identify the serotype by IFA tests using multi- and single-serotype-reactive MAbs.

Materials and methods

- FMDV strains were prepared in BHK-21 and IB-RS-2 cells.
- MAbs were raised against FMDV O/JPN/2000, A15 TAI 1/60 and Asia1 Shamir strains.
- The reactivity of the MAbs to several FMDV strains was analyzed by IFA tests.
- FMDV O/JPN/2000 and Asia1 Shamir strains were inoculated onto IB-RS-2 cells with cover slips at MOI 1 or 10^{-4} . The cover slip was collected periodically after the inoculation and examined for the appearance of fluorescent stained cells by the IFA tests as well as CPE.
- FMDV Asia1 Shamir strain was inoculated into three pigs and the plasma was collected periodically after the inoculation. The plasma samples were inoculated onto IB-RS-2 cells with cover slips and examined the appearance of fluorescent stained cells and CPE. They were also examined by RT-PCR and real-time RT-PCR assays.

Character of MAbs

Strains ^a MAbs		Strains							
		Type O				Type A		Type C	Type Asia1
		O/JPN/2000	O/Taiwan/97	O1 BFS 1860	O1 Manisa	A15 TAI 1/60	A22 IRQ 24/64	C PHI 7/84	Asia1 Shamir
O/JPN/2000	1H5	Positive	Positive	Positive	Positive	Positive	Positive	Positive	
	71F2	Positive	Positive	Positive	Positive	Positive	Positive	Positive	
	65H6	Positive	Positive	Positive	Positive	Negative	Negative	Negative	
	70C4	Positive	Positive	Positive	Positive	Negative	Negative	Negative	
A15 TAI 1/60	11H3	Positive	Positive	Positive	Positive	Positive	Positive	Positive	
	16C6	Negative	Negative	Negative	Negative	Positive	Positive	Negative	
Asia1 Shamir	7C2	Negative	Negative	Negative	Negative	Negative	Negative	Positive	

a: Strains of origin



Positive

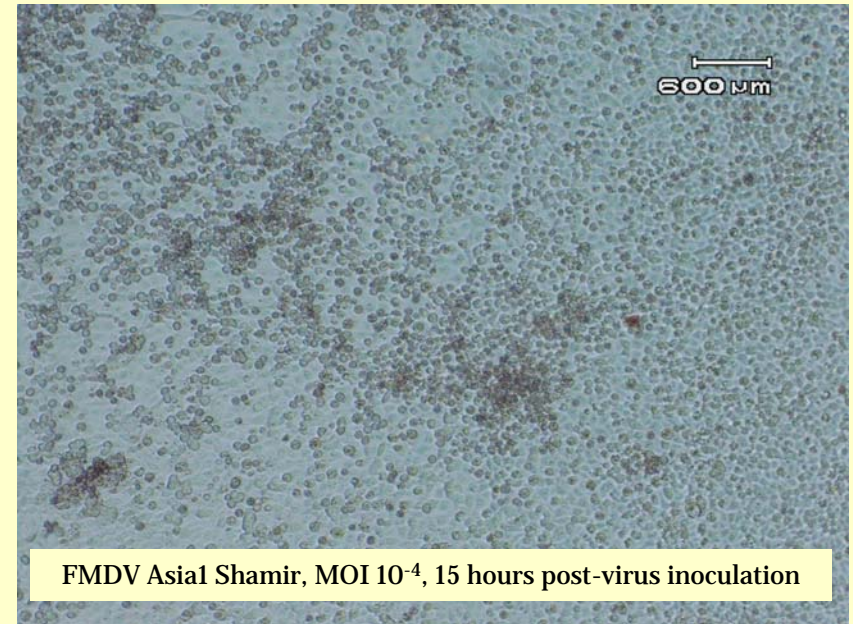
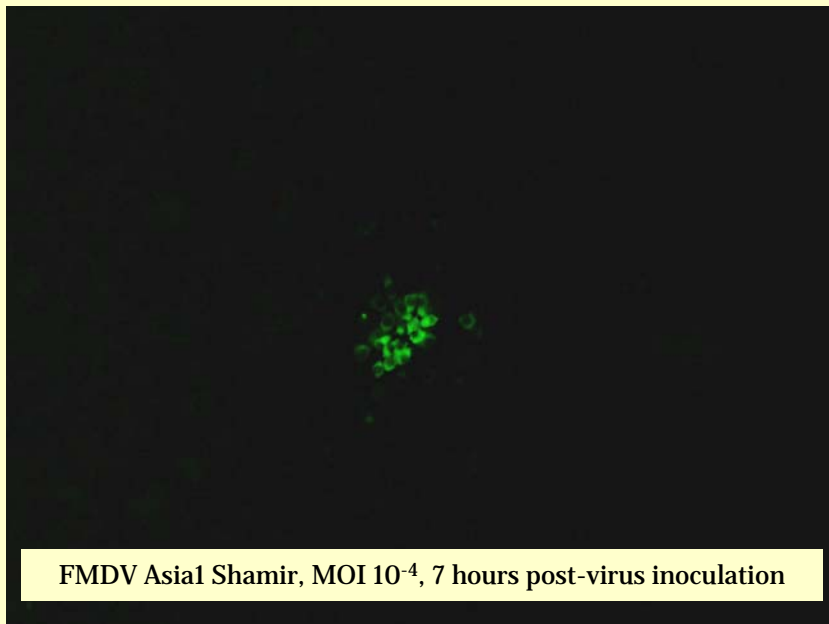


Negative

Detection of FMDV strains by using IFA tests and CPE observation

Strains	MOI	Methods	Hours post-inoculation																	
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
O/JPN/2000	1	IFA	-	+	+	+	+	+	+	+	+	+	+	NT ^a	NT	NT	NT	NT	NT	NT
		CPE	-	-	-	-	-	-	-	-	+	+	+	NT	NT	NT	NT	NT	NT	NT
	10 ⁻⁴	IFA	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
		CPE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
Asia1 Shamir	1	IFA	-	+	+	+	+	+	+	+	+	+	+	NT	NT	NT	NT	NT	NT	NT
		CPE	-	-	-	-	-	-	-	-	+	+	+	NT	NT	NT	NT	NT	NT	NT
	10 ⁻⁴	IFA	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
		CPE	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+

a: Not tested



Detection of FMDV from the plasmas of experimentally inoculated pigs by IFA tests

Days post-virus inoculation	Pig 1				Pig 2				Pig 4			
	IFA	CPE	RT-PCR	real-time RT-PCR	IFA	CPE	RT-PCR	real-time RT-PCR	IFA	CPE	RT-PCR	real-time RT-PCR
0	-	-	-	-	-	-	-	-	NT ^b	NT	NT	NT
1	-	-	-	-	+	+	+	+	-	-	+	+
2	+	+	-	+	+	+	+	+	+	+	+	+
3	+	+	+	+	-	-	+	+	+	+	+	+
4	-	-	+	+	-	-	-	+	-	-	-	-
5	-	-	-	+	-	-	-	+	-	-	-	-
6	-	-	-	-	-	-	-	+	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-

a: The days when fluorescent stained cells or CPE were detected after the plasma sample was inoculated onto IB-RS-2 cells.

b: Not tested

· When the plasma samples obtained from the experimentally inoculated pigs were inoculated onto IB-RS-2 cells with cover slips, the appearance of fluorescent stained cells was completely agreed with that of CPE.

Conclusion

- An IFA test has been successfully applied for the assay of both cytopathogenic and noncytopathogenic strains of many animal and human viruses.
 - A few researchers have reported the detection of FMDV by the IFA test using anti-FMDV sera obtained from the infected animals in 1970s and 1980s. However, the IFA test has hardly been evaluated as a diagnostic assay for FMDV.
 - In this study, we attempted to rapidly detect FMDV and identify the serotype by the IFA test using multi- and single-serotype-reactive MAbs.
 - We generated results earlier by the IFA test and the appearance of the fluorescent stained cells was completely agreed with that of the CPE.
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- These results suggest that the IFA test using multi- and single-serotype-reactive MAbs is rapid and useful antigenic assay for FMDV.