Development of mAbs against FMDV Asia 1 and application in rapid virus detection by lateral flow strip test

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- FMD remains one of the world's most widespread and highly contagious animal diseases.

- More than 100 countries are not yet recognized as officially free of FMD by the OIE.

- FMDV is recognized as seven serotypes: O, A, C, Asia 1, SAT 1, SAT 2 and SAT 3.

- Among the seven serotypes O and A are the most widespread.

- Asia 1 has been rapidly spreading across Asia, periodically into the Middle East and occasionally to Europe.

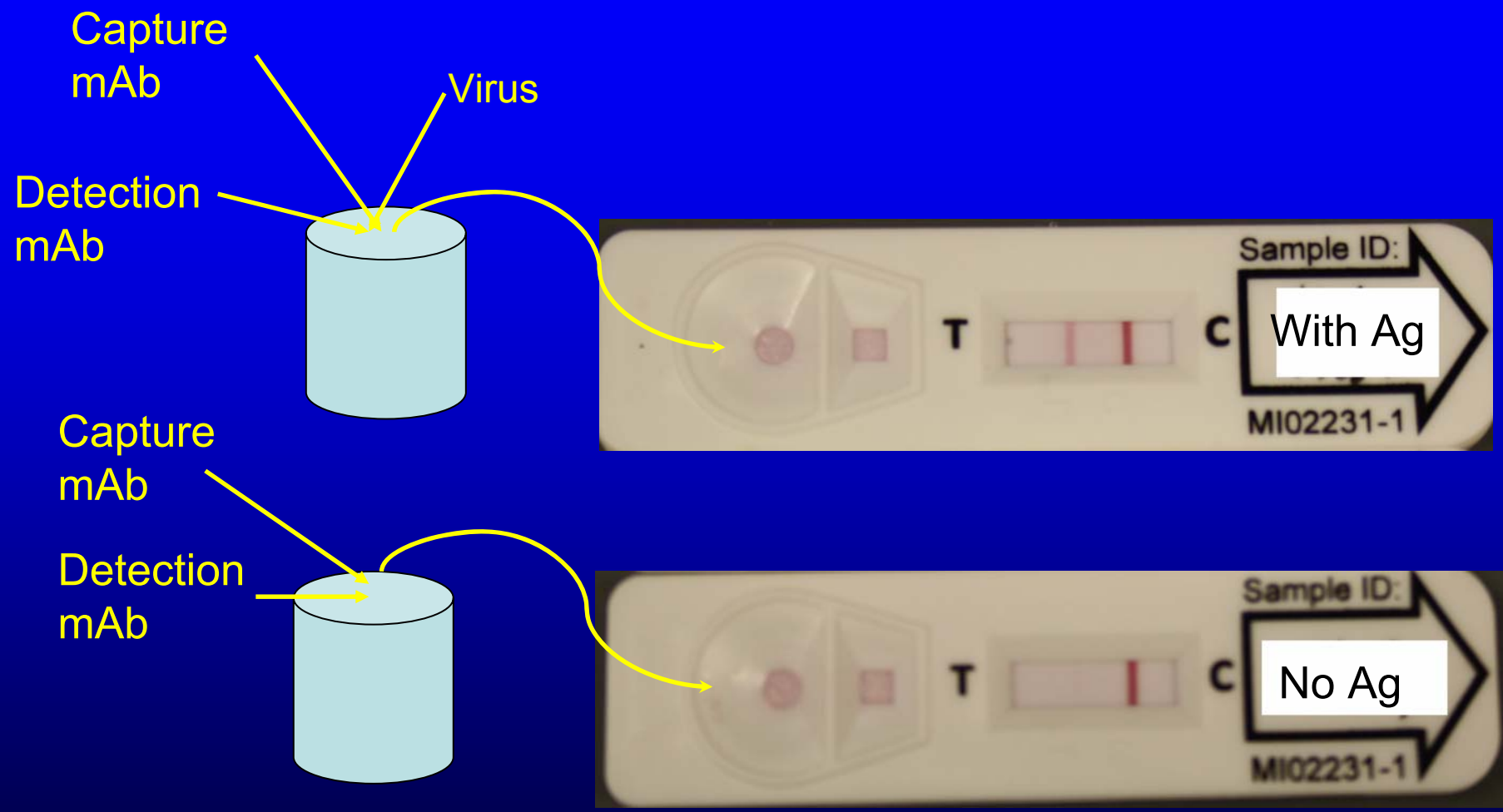
- Eighteen countries have reported FMDV Asia 1 outbreaks from 2000 to 2008.

Methods are available for FMDV detection:

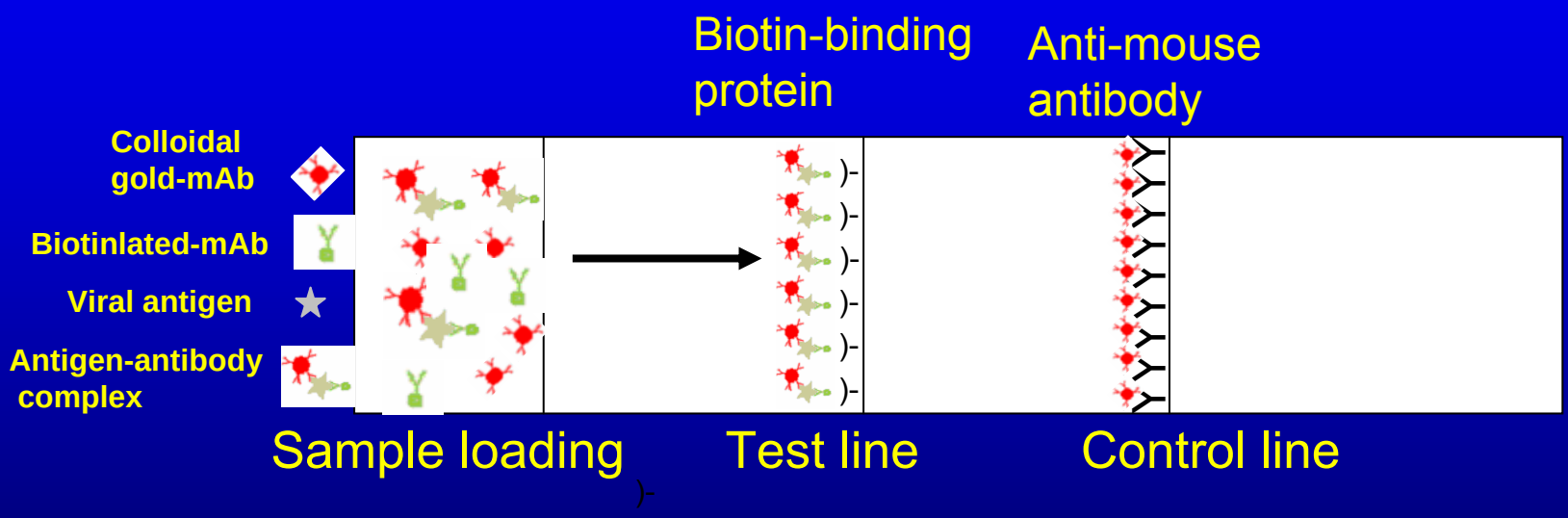
* Virus isolation
* Real-time RT PCR
* Double antibody sandwich ELISA

Advantages of the lateral flow strip test:

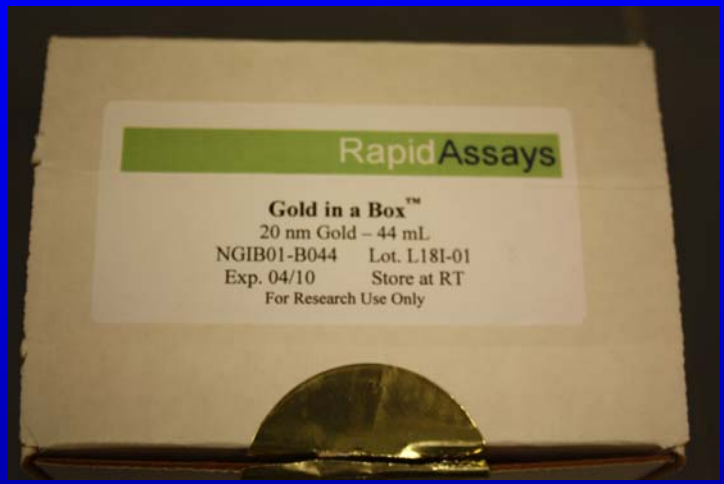
*Low cost
*Short timeline for development
*Ease of performing and result interpretation
* No requirement for trained personnel and special equipment


Capture mAb
Virus
Detection mAb
T
C
Sample ID:
With Ag
MI02231-1
Capture mAb
Detection mAb
T
C
Sample ID:
No Ag
MI02231-1

Rapid Assay Device



Colloidal-gold mAb conjugate



- Colloidal gold is a suspension of nanometer sized gold particles
- The liquid is usually an intense red colour.
- Proteins bind to gold particles through both ion-exchange attraction and covalent bond.
- The binding of a antibody to gold particles is irreversible.

Table I. Characterization of mAbs raised against FMDV Asia 1 serotype

MAbs	Isotype	Epitope	VNT (Asia 1 Shamir)
F34-8	IgG2a/k	Conformational	+
F36-14	IgG1/k	Conformational	-

MAb reactivity with FMDV seven serotypes in a DAS ELISA

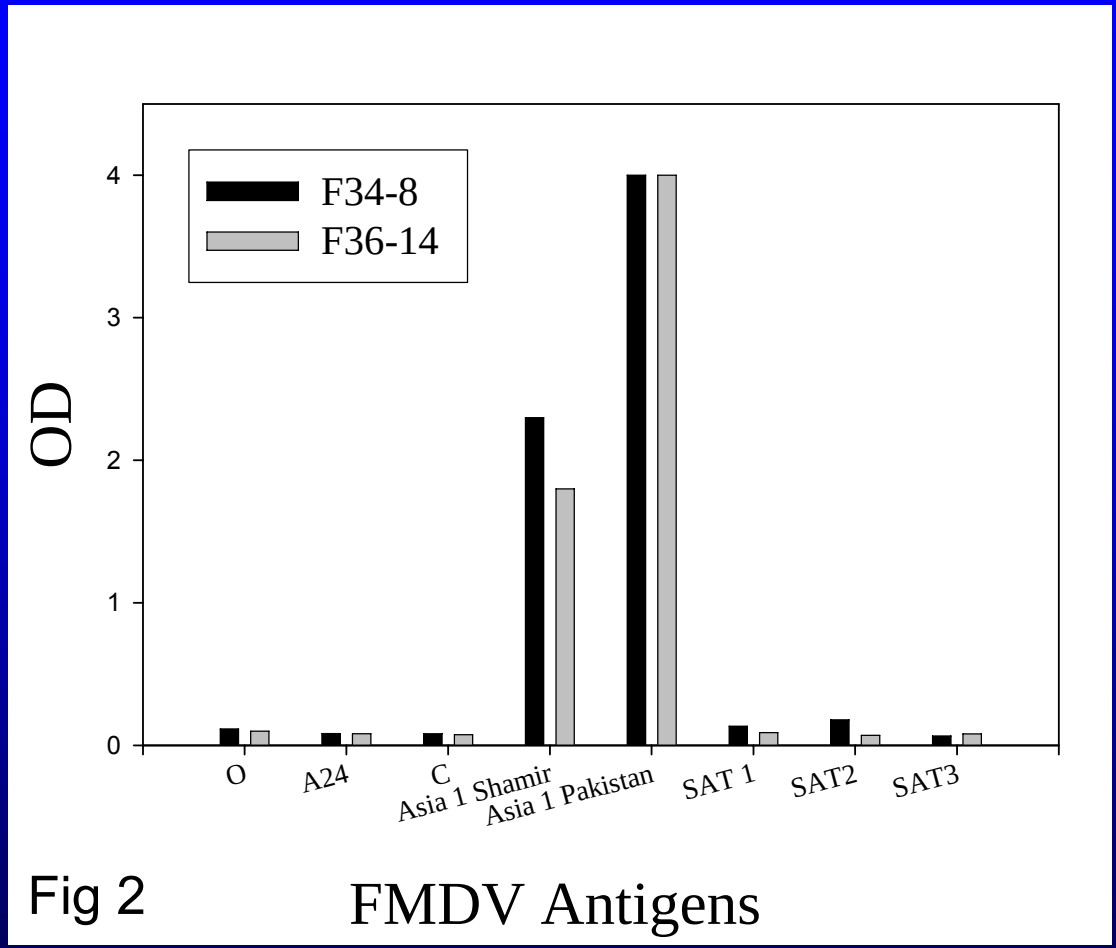


Fig 2

Polyclonal rabbit anti-FMDV seven serotype antibodies were coated onto microtiter plates as the capture Abs. Then the FMDV seven serotype antigens were added to the plates and detected with two Asia 1 specific mAbs

Characterization of mAbs' binding sites using an ELISA

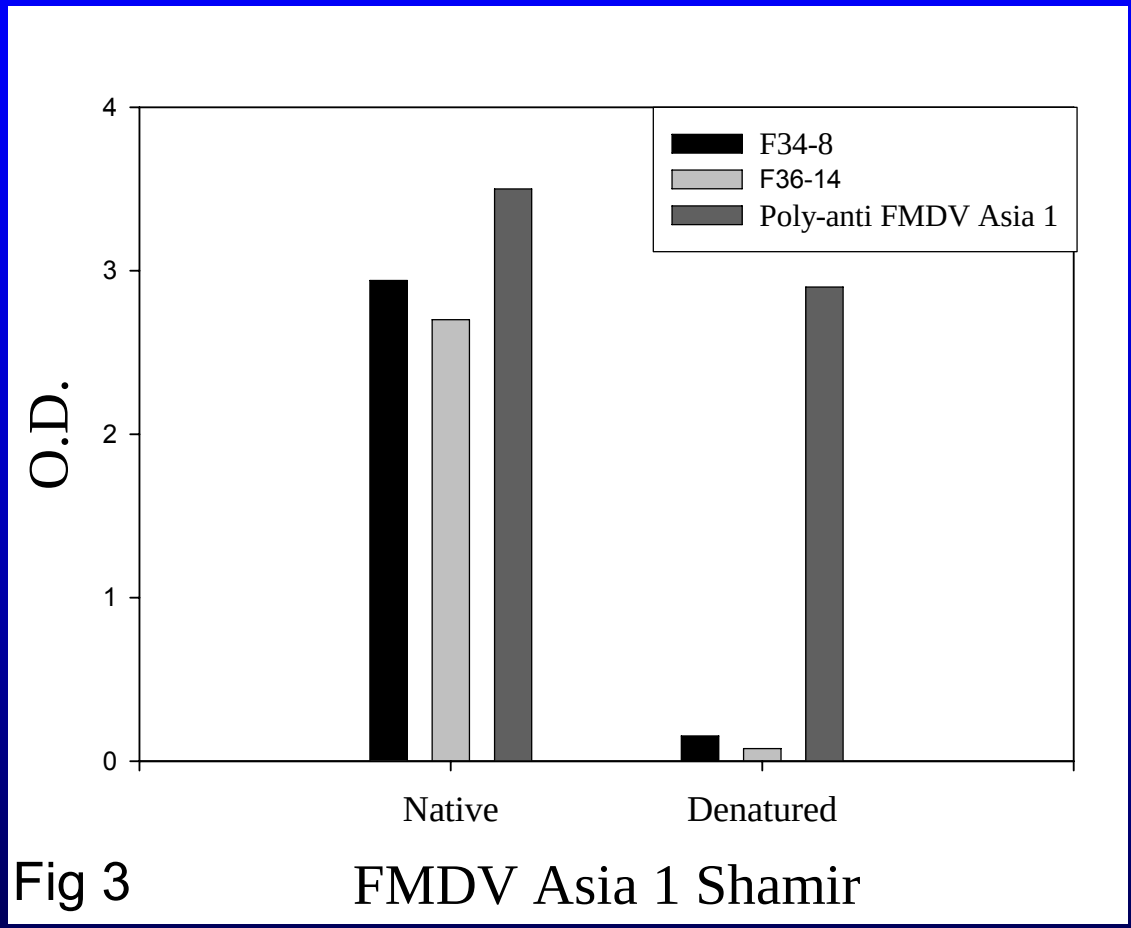


Fig 3

Purified native and denatured FMDV Asia 1 Shamir antigens were coated onto microtiter plates. Then two Asia 1 specific mAbs and a polyclonal serum against FMDV Asia 1 were added. The binding was detected with an HRP anti-mouse IgG.

MAb reactivity with different Asia 1 strains in a DAS ELISA

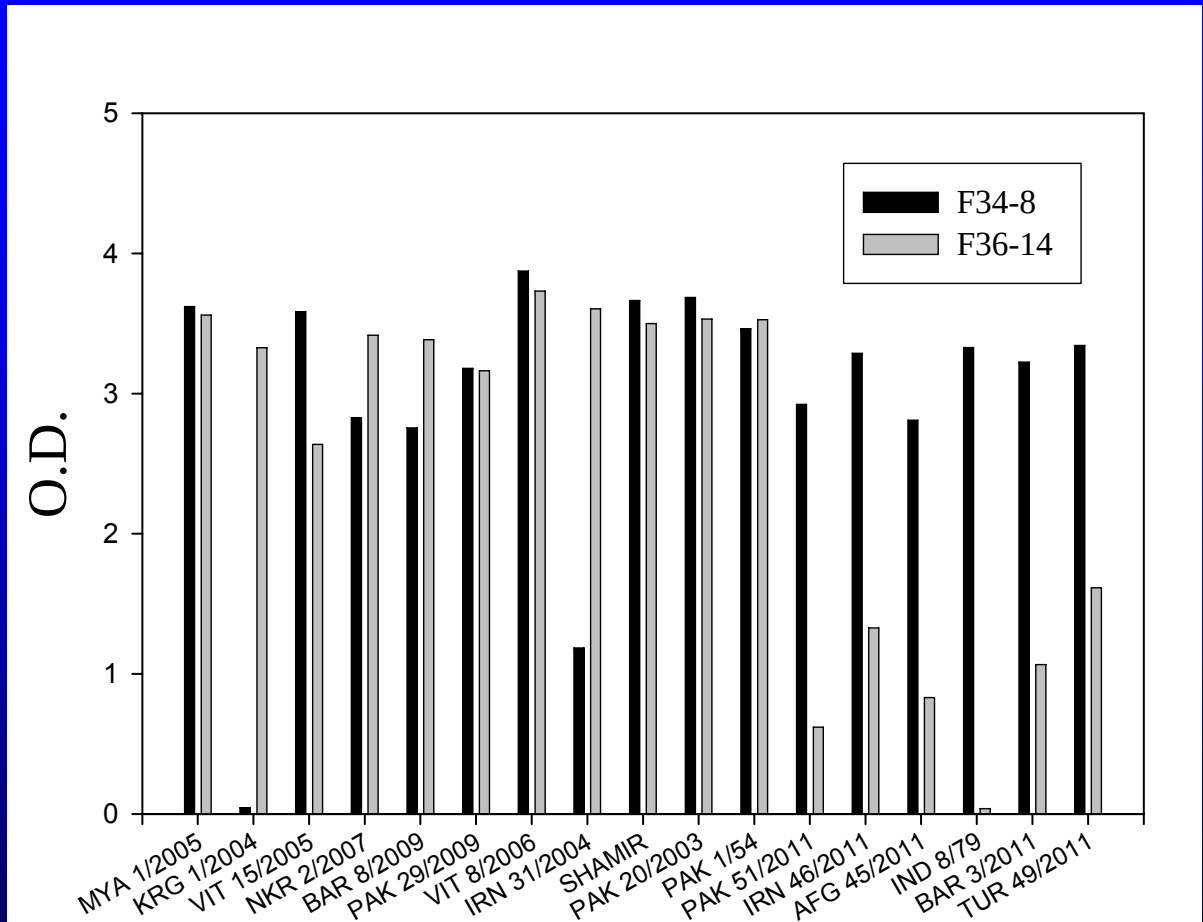


Fig 4 FMDV Asia 1

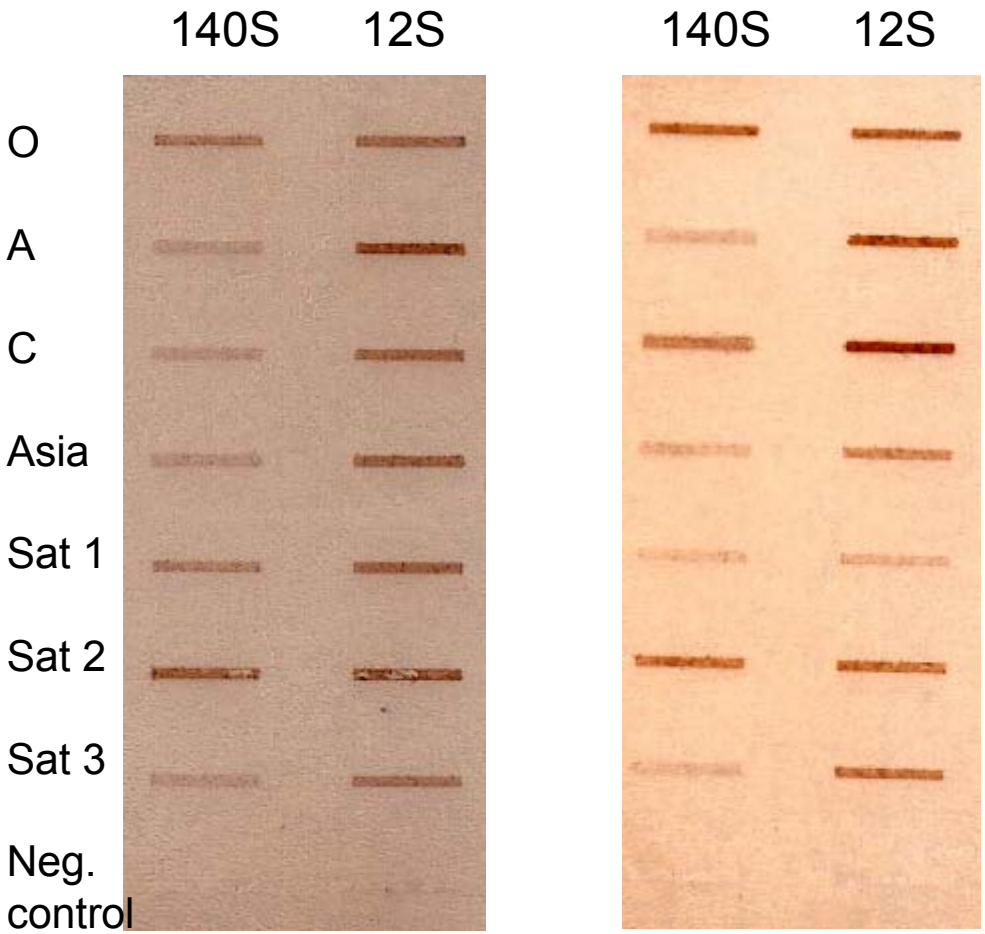
A polyclonal rabbit anti-FMDV Asia 1 antibody was coated onto microtiter plates. Then seventeen strains of FMDV Asia 1 were added to the plates and detected with two Asia 1 specific mAbs

Table 1. Characterization of monoclonal antibodies raised against FMDV

Clones	Immunization Ag	Isotype	Western blot	Epitope
F1412SA	FMD-A-12S	IgG1/k	+	Linear
F21140SO	FMD-O-140S	IgG1/k	-	Conformational

Yang et al., Veterinary Immunology and Immunopathology 115 (2007) 126–134

Slotblot analysis of MAbs reactivity with FMDV



Seven serotypes of FMDV proteins and a negative control supernatant were blotted onto NC membrane. The proteins were detected using MAbs

Selection of detection mAbs using a DAS ELISA

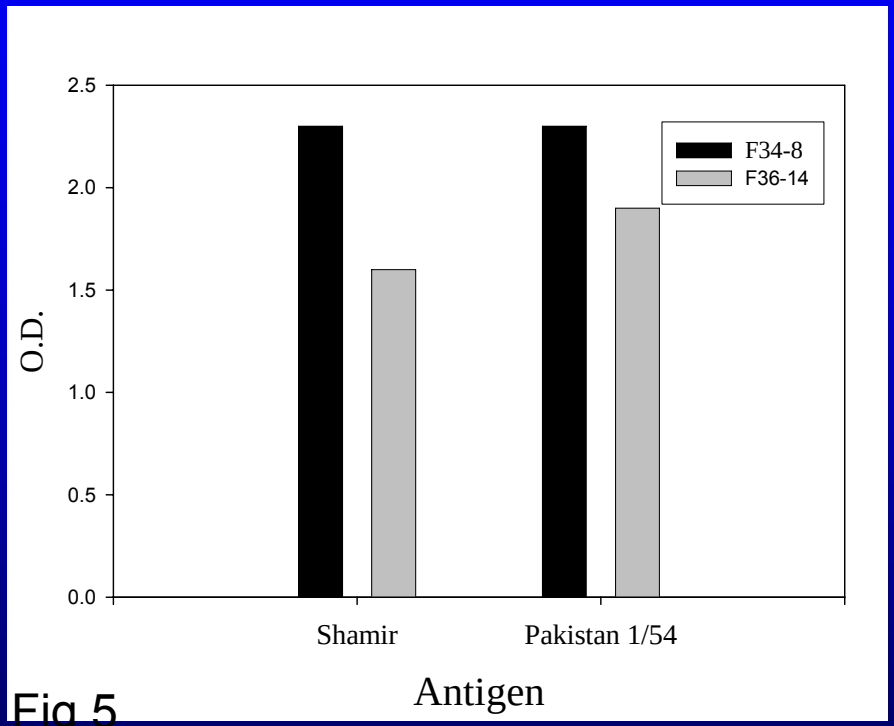
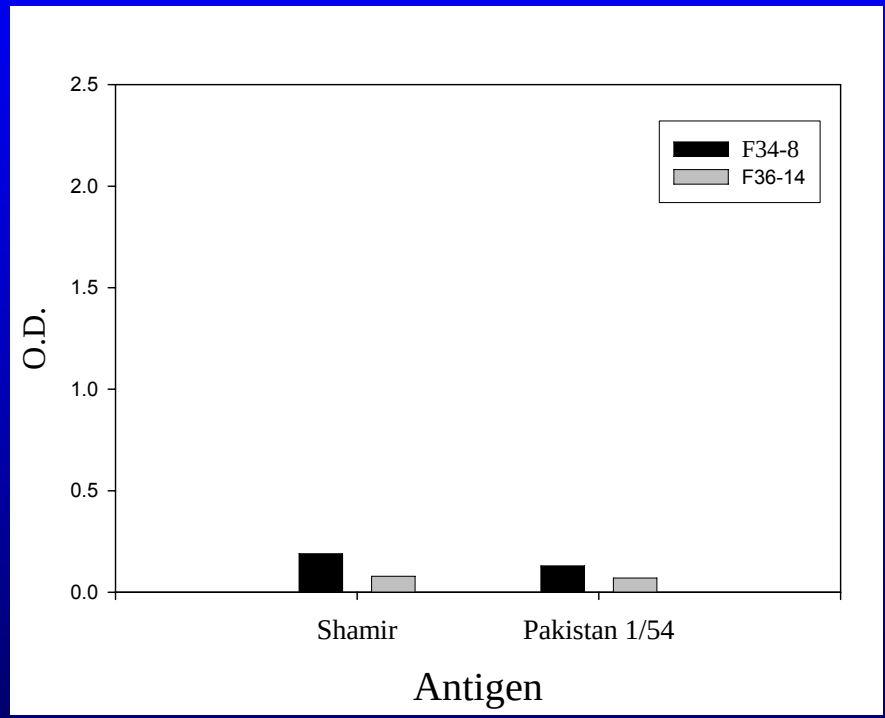


Fig 5



Two purified mAbs (F34-8 and F36-14) were coated onto microtiter plates. Then the FMDV Asia 1 Shamir and Pakistan 1/54 antigens were added to the plates. The antigen binding was detected with biotinylated serotype independent mAbs: (a) mAb F21-140SO and (b) mAb F14-12SA. The binding was detected with an avidin-HRP

Table II. Comparison of different sizes of colloidal-gold particle Conjugated detection mAb in the strip test

Gold particle size	20 nm		40 nm		60 nm	
Antigen dilution	ESE reading	Visible band	ESE reading	Visible band	ESE reading	Visible band
1:16	109.93	+	244.52	++	226.84	++
1:32	63.07	+	174.40	+	237.03	++
1:64	31.57	+	95.69	+	142.10	++
1:128	16.14	-	43.23	+	88.09	+
1:256	18.79	-	19.21	-	25.43	+

+ +: Strong band; +: Light band; -: No visible band.

Specificity of the strip test

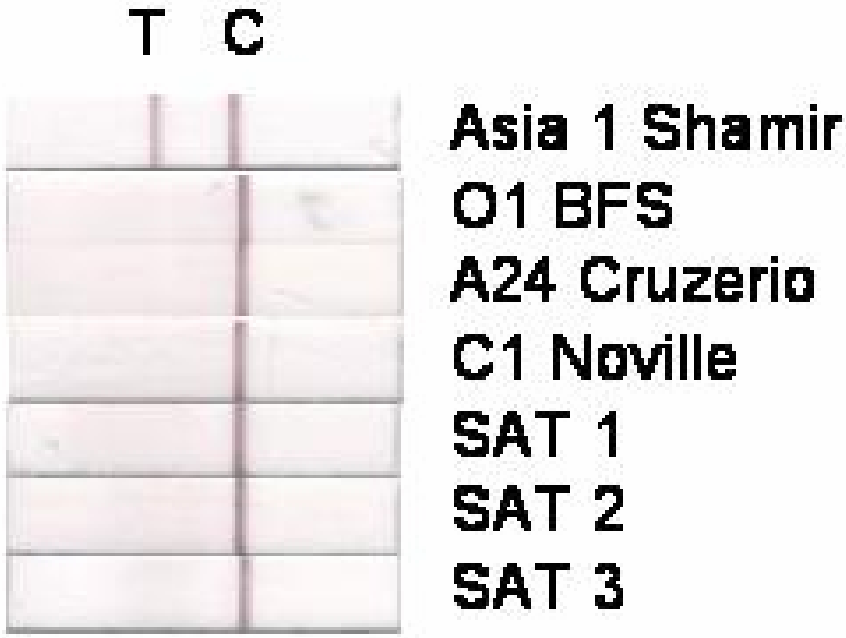


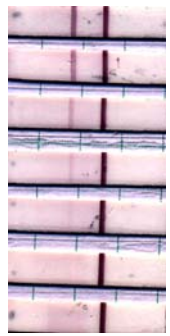
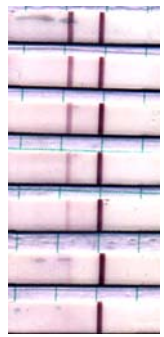
Fig 6

Culture supernatants containing the seven serotypes of FMDV (50 μl) was mixed with 1 μl of each biotinylated F34-8, F36-14 and 6 μl of colloidal-gold conjugated F21-140SO in the running buffer. The mixture (100 μl) was applied to the gRAD.

Table III. The strip test results for FMDV seven serotypes and other vesicular disease viruses

Virus	Strip test results/Sample tested (total n=149)
FMDV Asia 1	17/17
FMDV O	0/40
FMDV A	0/37
FMDV C	0/9
FMDV SAT 1	0/11
FMDV SAT 2	0/19
FMDV SAT 3	0/4
SVDV	0/4
VSV (IN&NJ)	0/6
SVV	0/1
VESV	0/1

Table IV. Comparison of strip test with DAS ELISA for tissues collected from experimentally inoculated pigs with FMDV Asia 1

Tissue	Sample dilution	Viral titer log10 TCID50/ml	DAS ELISA OD	DAS ELISA Results	Strip test Result	Strip test Picture
Interdigital	1:2	6.8	1.45	+	++	
	1:4	6.3	1.069	+	++	
	1:8	6.0	0.467	+	+	
	1:16	5.7	0.236	+	+	
	1:32	5.4	0.167	+	±	
	1:64	5.1	0.035	-	-	
	1:128	4.8	0.017	-	-	
Coronary Band	1:2	7.8	1.989	+	++	
	1:4	7.5	1.65	+	++	
	1:8	7.2	1.196	+	++	
	1:16	6.9	0.746	+	+	
	1:32	6.6	0.379	+	+	
	1:64	6.3	0.177	+	±	
	1:128	6.0	0.055	-	-	

Strip test results for different FMDV Asia 1 strains

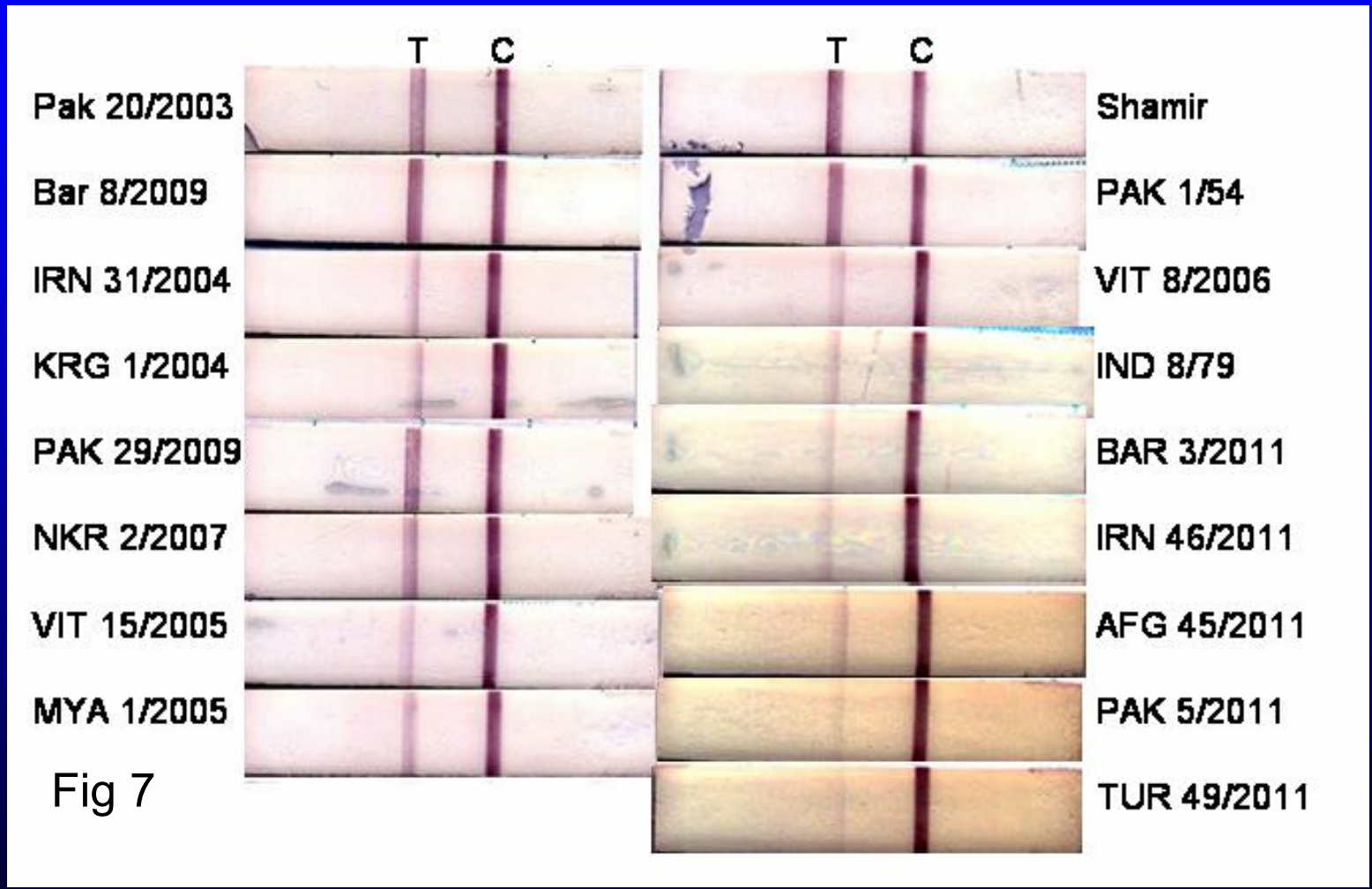


Fig 7

Table V. FMD Asia 1 virus detection using different methods for samples collected from experimentally inoculated animals

Samples	Dpi	StripTest	DAS ELISA	Real time PCR	Viral titer (log10TCID50/ml)
Vesicular fluid	3-4	4/4	4/4	4/4	6.06-8.18
Tongue	4	2/2	2/2	2/2	5.3-5.5
Coronary band	3-4	4/4	4/4	4/4	6.3-7.8
Interdigital area	3-4	4/4	4/4	4/4	5.05-7.66
Serum	3-4	1/4	0/4	3/4	NA
Nasal swab	1	0/4	0/4	1/4	NA
Nasal swab	2	0/4	2/4	3/4	NA
Nasal swab	3	0/4	1/4	4/4	NA
Nasal swab	4	0/1	1/1	0/1	NA

Samples collected from experimentally inoculated pigs with FMDV Asia 1 were analyzed using the strip test, the FMD Asia 1 DAS ELISA and the RRT PCR.

Summary

1. The two capture mAbs and one detection mAb with high binding affinity to FMDV Asia 1 are crucial to the high specificity and sensitivity requirement for the strip test development.

2. A strip test for FMDV serotype Asia 1 was developed and achieved sensitivity similar to the DAS ELISA.

3. This newly developed strip test is suitable for rapidly detection of large number samples on-site during a major epidemic.

4. The gRAD and the colloidal gold-conjugated serotype Independent detection mAb are suited for different serotype strip tests without modification of the device.

Acknowledgement

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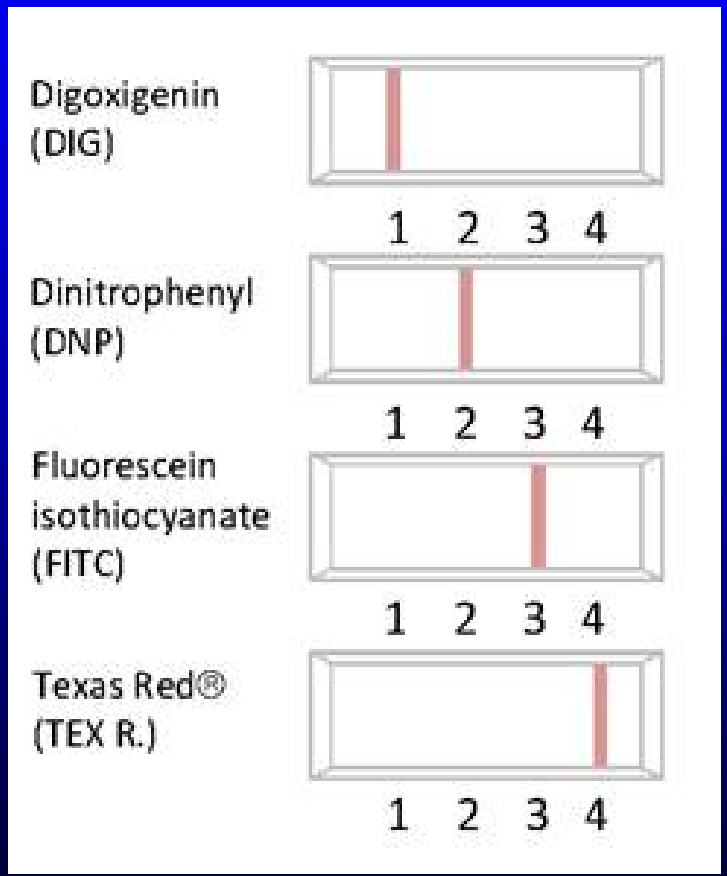
Future work

gRAD-4 lateral flow device



* The gRAD-4 lateral flow device (LFD) allows to develop 4 tests on the one device.

* The different capture antibodies will be labelled with different tags. Antibodies specific to these tags are immobilised at the test lines and will immobilise the capture antibody.

* The Colloidal gold-detection antibody will form a complex with the Ag and the capture antibodies and make the test line visible.

Thank you for your attention

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