



Simplified & Stabilised FMD Diagnostic Kits



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SCIENTIFIC DEVELOPMENTS AND TECHNICAL CHALLENGES
IN THE PROGRESSIVE CONTROL OF FMD IN SOUTH ASIA



Aims

Generate “next generation” ready-to-use kits

For FMDV antigen detection / serotyping

- ✓ MAb based – sandwich ELISA
- ✓ Lateral flow devices (LFDs)

For antibody detection

- ✓ Ab to FMDV serotypes O, A, Asia 1 → MAb based – SPCE

Test Purposes

Antigen detection

- Rapid detection and serotyping of FMDV
- Direct testing of lesion materials
 - Vesicular epithelium or fluid
- Indirect testing of all samples after amplification in cell cultures

Structural protein serology

- Diagnosis of suspect cases
- Estimating prevalence and substantiating freedom
- Estimating vaccine efficacy and population immunity
- Trade certification

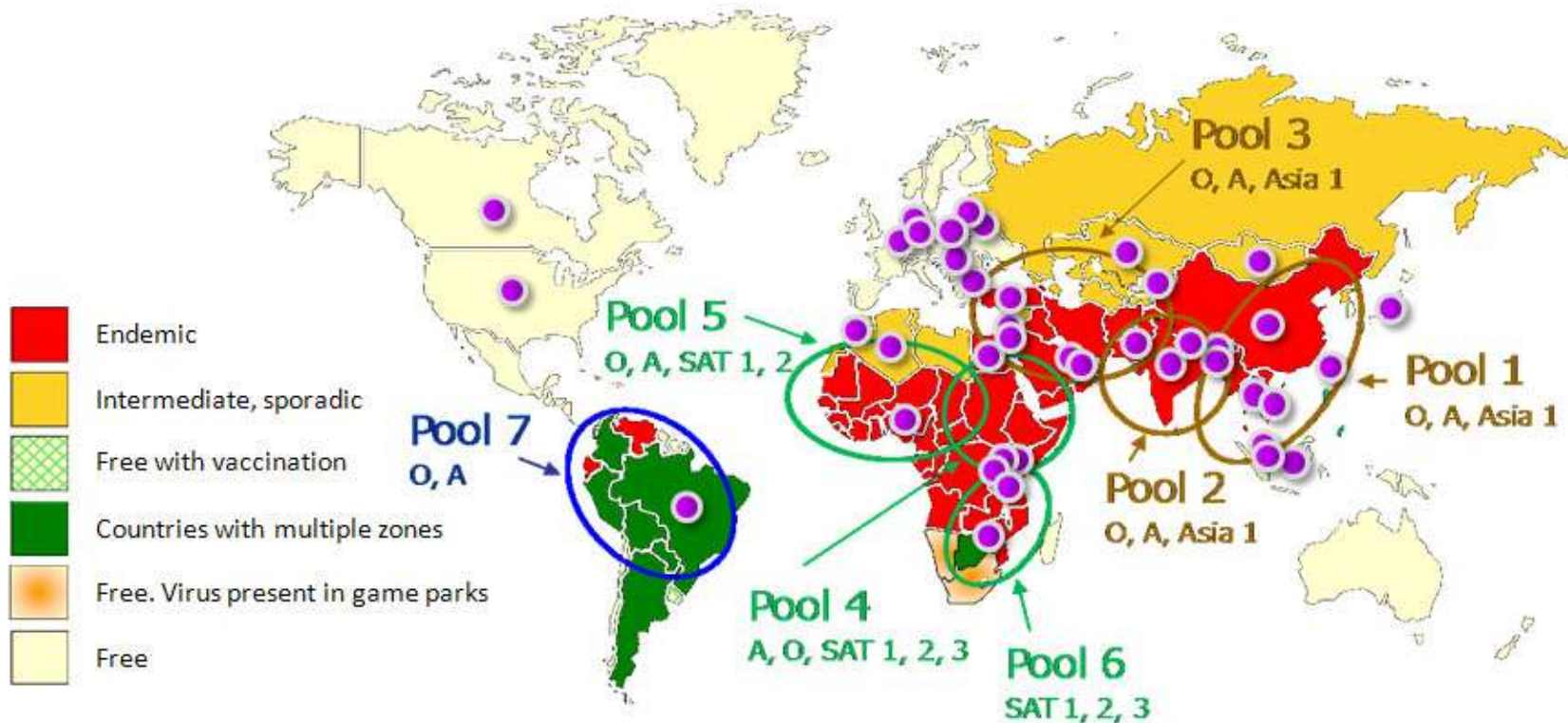


Current ELISA formats

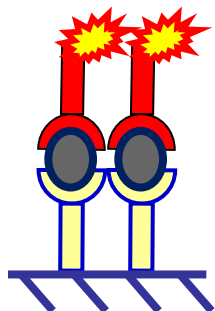
- Serotype specific sandwich ELISA kits (antigen detection)
 - Hamblin et al Vet Microbiol (1984)
 - Rabbit polyclonal capture antibody
 - Guinea pig polyclonal indicator antibody
 - Control antigens are inactivated cell culture grown virus
- Serology ELISA kits (antibodies to structural proteins)
 - LPBE - Hamblin et al J Immunol Methods (1986)
 - SPCE - Have & Jensen Res Group Proceedings (1983)
 - Some alternative simplified tests using mAbs

Supply of FMD ELISA kits in 2011

● FMD diagnostic kits and reagents supplied by IAH Pirbright



FMDV antigen detection/serotyping kit sandwich ELISA – Monoclonal Antibodies



DETECTOR MAb PO-conjugated

FMDV Antigen (positive sample)

CATCHING MAb (coated)

Catching MAb	Unique conjugated MAb pan-FMDV	FMDV specificity
type O	1F10	O
type A	1F10	A
type Asia 1	1F10	Asia 1
Type C	1F10	C
pan-FMDV	1F10	FMDV



- ✓ **Stable at 5°C (> one year)**
- ✓ **FMDV control antigens already present in relevant wells**
- ✓ **All stabilized reagents included (conjugates, buffers, chromogen)**



Selection of typing MAbs

FMDV Serotype	N. isolates tested	Period covered	Final selection of catching Monoclonal Antibodies							
Type A	130	↓ 1943 2010	MAbs N. isolates reactive	4D12 122	5F6 112	1C11 113				
Type O	108	↓ 1950 2010	MAbs N. isolates reactive	3B11 105	A8 105	7E1 105	3C8 81			
Type Asia 1	53	↓ 1954 2009	MAbs N. isolates reactive	3D8 53	1F10 51	4G6 48	3C6 43	5E10 35		
Type C	33	↓ '60s 2005	MAbs N. isolates reactive	3E9 32	2B1 32	5C4 32	4D7 31	2E5 30	3E5 27	4C4 27

Results & Interpretation

		Type O		Type A				Type Asia 1		Type C		Pan-FMDV	
		MAb 3B11		MAb 4D12		MAb 5F6		MAb 3D8		MAb 3E9		MAb 1F10	
		1	2	3	4	5	6	7	8	9	10	11	12
Sample 1	A												
Sample 2	B												
Sample 3	C												
Sample 4	D												
Sample 5	E												
Sample 6	F												
POS contr	G												
NEG contr	H												

Pos O



Results & Interpretation

		Type O		Type A				Type Asia 1		Type C		Pan-FMDV		SAT ?
		MAb 3B11		MAb 4D12		MAb 5F6		MAb 3D8		MAb 3E9		MAb 1F10		
		1	2	3	4	5	6	7	8	9	10	11	12	
Sample 1	A													
Sample 2	B													
Sample 3	C													
Sample 4	D													
Sample 5	E													
Sample 6	F													
POS contr	G													
NEG contr	H													



Sensitivity

FMDV types	N.	Type-specific MAbs-based ELISAs		Polyclonal ELISA		Pan-FMD ELISA based on MAb 1F10	
		POS	Sens.	POS	Sens.	POS	Sens.
O	136	116	85%	129	95%	118	87%
A	103	67	65%	41	40%	67	65%
Asia	30	22	76%	26	90%	21	72%
C	29	19	66%	20	69%	20	69%
TOTAL Epithelium suspensions	298	224	75%	216	72%	226	76%



FMDV antigen detection/serotyping

Evaluation on field samples

External evaluation (field samples)

- Kimron Veterinary Institute, Israel (April 2011)
- Indian Immunologicals Limited , India (October 2011)
- SAP Institute, Turkey (May 2011)

Demonstration/training (*Kees van Maanen*)

- Kenya
- Egypt

Supply (FAO, EUFGMD programs)

- Afghanistan, Pakistan, Tajikistan
- Iran, Armenia, Azerbaijan, Georgia

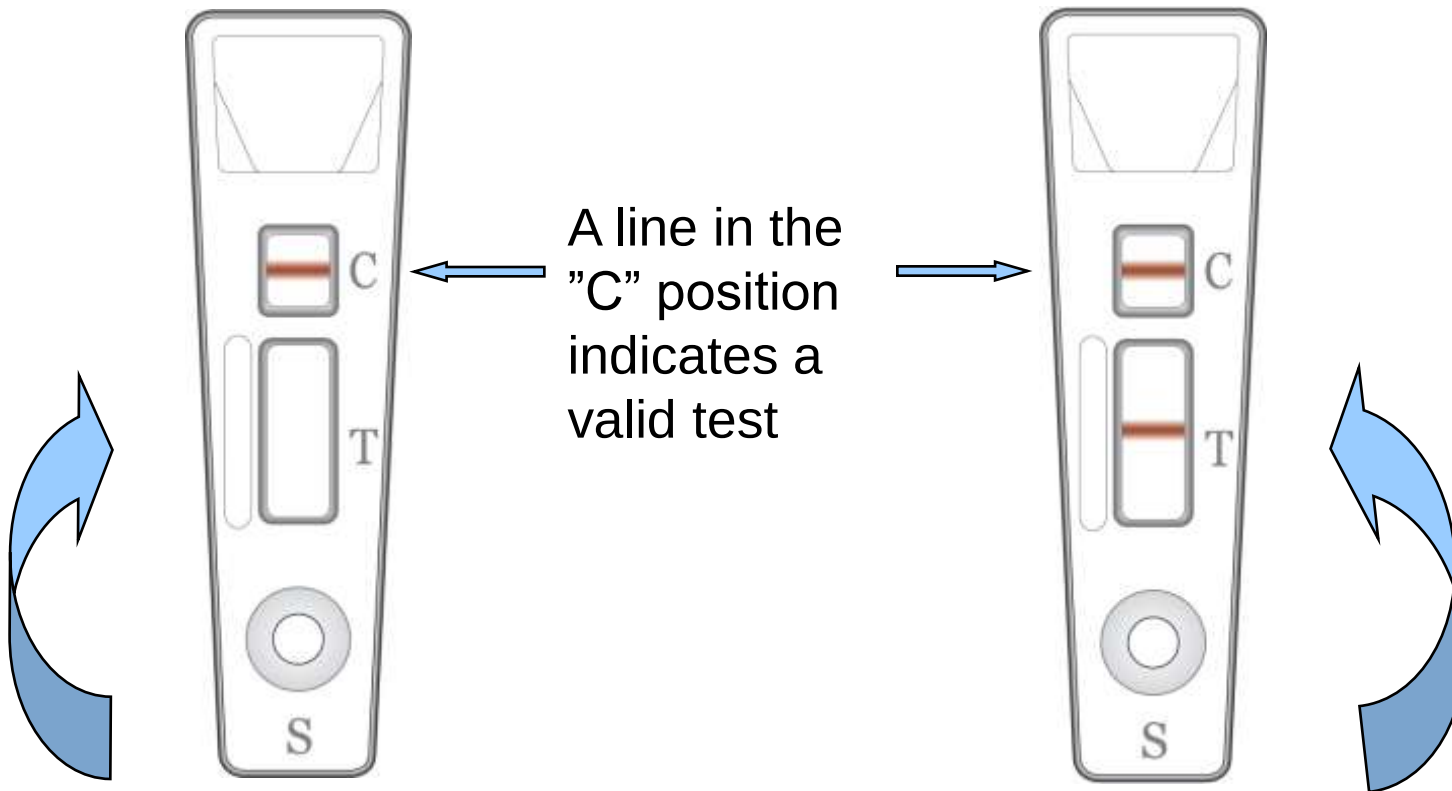
Work in progress:

Development of similar kits for FMDV SATs

- At IZSLER: several MAbs specific for SAT 1 and SAT2
- At IAH : several MAbs specific for the three SAT serotypes
- Identified at least two candidates MAbs for each serotype
- Candidate MAbs purified and peroxidase-conjugated
- Sandwich ELISAs with anti-SAT 1, SAT2, SAT3 selected MAbs
- Specific and efficient reactivity with reference viruses

LFD Test Procedure

Results read after 10-30 minutes



No line in the "T" position:

NEGATIVE test result

A line in the "T" position:

POSITIVE test result



LFD Development and Application

- Pan-specific test (based on pan-FMDV Mab 1F10)
 - Ferris et al J Virol Methods (2009)
 - Used in UK 2007 outbreaks
 - Sammin et al Transbound Emerg Dis (2010)
- SAT2 specific test (based on a pan-SAT2 specific Mab)
 - Ferris et al J Virol Methods (2010)
- Asia 1 specific test (based on a pan-Asia1 specific Mab)
 - Validated on 160 epithelium samples at IAH
 - Available through EUFMD
- Other type specific tests
 - Under development



ELISA Kits for SP-Ab detection FMDV serotypes O, A, ASIA 1

Objective: convert in-house MAbs based SPCE-ELISA into “ready to use” ELISA kits

Desired properties for these kits:

✓Fast and easy

- All inclusive
- Use of a single protocol for the three different serotypes
- Test run in only 2-2.5 hours (Room Temperature)
- Specific skill not required

✓Stable

- Shelf-life $\geq$ 1 year

✓Reliable

- Diagnostic performance under evaluation





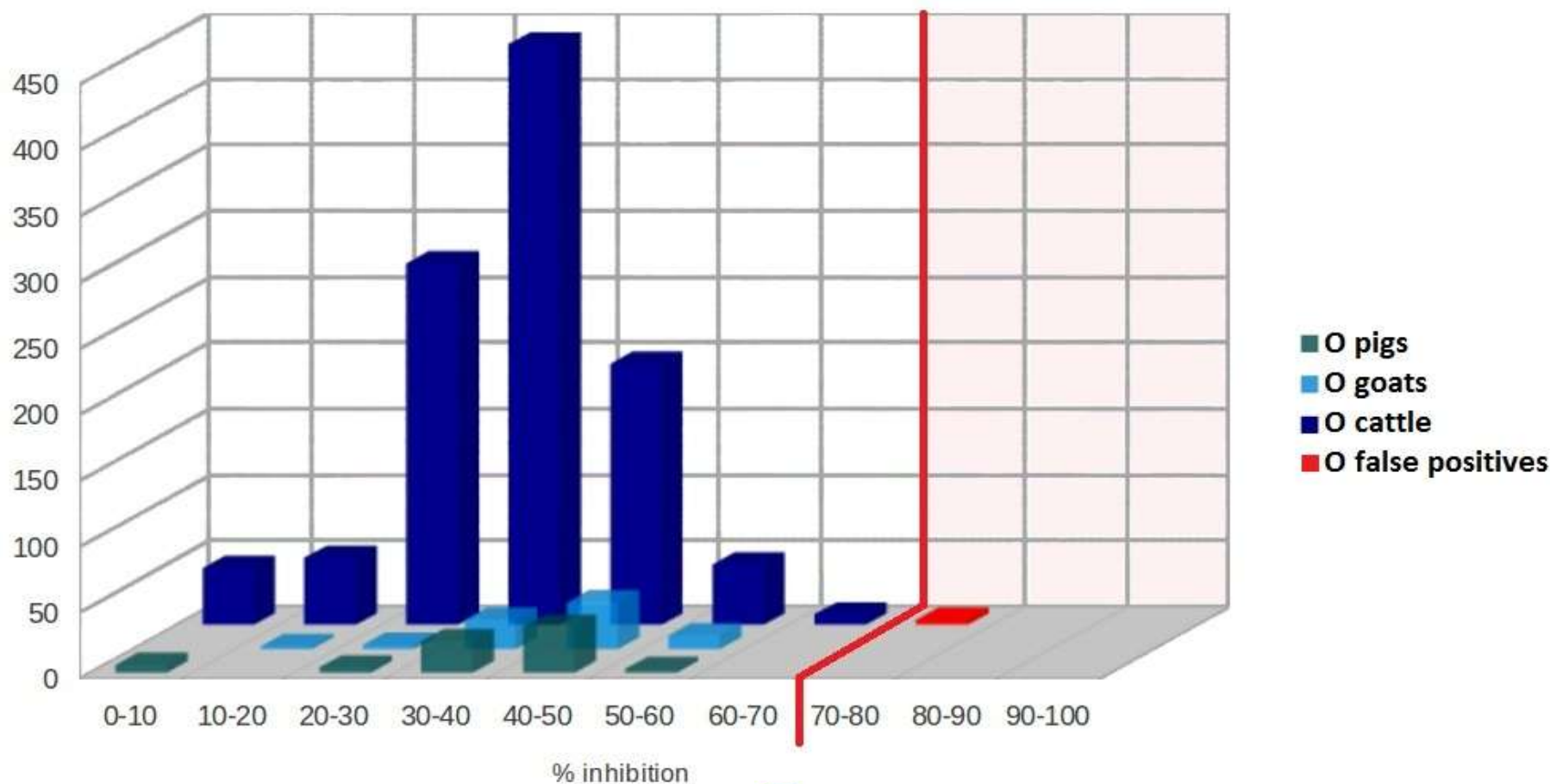
Starting point → in-house SPCE, MAbs-based

- ✓ Conditions for stabilization of inactivated FMDV antigens trapped by homologous MAbs defined
 - >1 year the shelf-life of plates sensitized with FMDV captured by MAbs
- ✓ Conditions for stabilization of conjugated MAbs (competition phase) defined
 - shelf-life ranging from 1,5 to 3 years
- ✓ Drawback : FMDV antigens tend to degrade to 12S during the stabilization process → need of initial excess of reagents
- ✓ Prototype kits developed and under evaluation
- ✓ Possible improvement: use of recombinant capsid particles with stabilized structure



Test results: FMDV O (strain O Manisa)

- ✓ 1064 naive cattle sera: 4 false positive & 4 borderline = **0,75% FP**
- ✓ 72 naive pig sera: 0% false positive
- ✓ 72 naive goat sera: 0% false positive





Test results: FMDV O (strain Manisa)

104 sera from 7 vaccine potency tests
(courtesy of CODA-CERVA)

Vaccine dose	Number sera	N. POSITIVE			
		VNT	in-house LPBE	Kit SPCE	in-house SPCE
1	35	35 (100%)	32 (91%)	35 (100%)	11 (31%)
1/4	35	26 (74%)	12 (34%)	17 (48%)	2 (6%)
1/16	34	12 (35%)	3 (9%)	6 (17%)	0 (0%)

Next steps

- ✓ Wider *Se* and *Sp* validation underway for each FMDV serotype: **O**, **A**, **ASIA1**
- ✓ External evaluation and completion of validation
- ✓ Analyze field sera from endemic countries
- ✓ Refine the reaction setup, if necessary
- ✓ Start the setup of ELISA kit for FMDV SAT 1 and SAT 2 serotypes



Conclusions

- ✓ Pan-specific, SAT2-specific and Asia1-specific LFDs launched
- ✓ New antigen detection ELISA for Eurasian FMD ready for launch
- ✓ Eurasian serotype-specific Ab ELISAs under final validation
- ✓ SAT-specific Ab/Ag ELISAs under development
- ✓ Other serotype-specific LFDs to follow

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