

# Progress Toward the development of a safe antigenic marker Foot-and-Mouth Disease Vaccine Platform



**Elizabeth Rieder PhD.**

Foreign Animal Disease  
Research Unit, USDA-ARS  
Plum Island Animal Disease Center, New York, USA.



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# Characteristics of an “Ideal” FMD Vaccine

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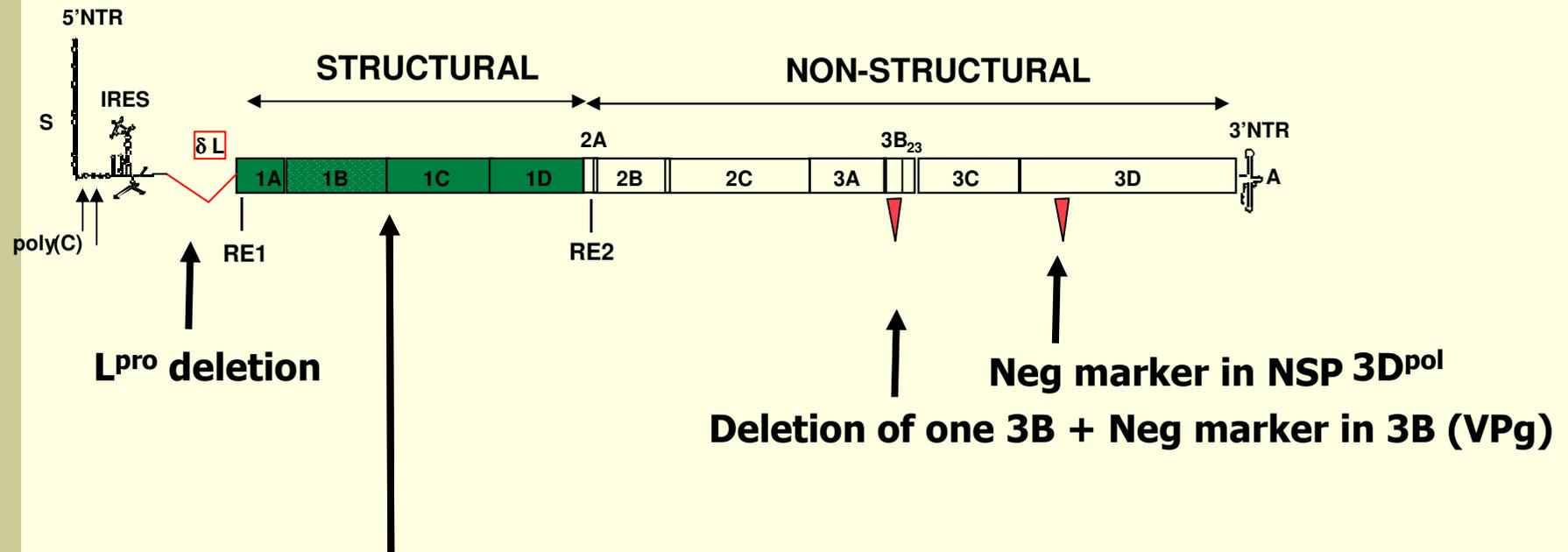
- Effective, rapid and **long-lasting** protection with one inoculation
- Prevents viral transmission
- Allow differentiation of infected from vaccinated animals (DIVA)
- Produced without the need for virulent FMDV
- Prevent development of carrier state
- Protection against multiple serotypes
- Stable antigen – long shelf life

# Concerns with FMD Vaccines in *Disease-Free* Countries

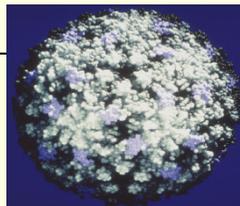
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- Require adaptation and growth of large volumes of wild type virus in cells
- Escape of virus from manufacturing facilities
- Require banking of multiple antigen concentrates
- Some antigens lack stability (low potency/short shelf life)
- Onset of protection 7-14 days
- Short duration of immunity  $\leq 6$  months
- Difficult to differentiate vaccinated from infected animals (DIVA) due to presence of NS proteins
- Vaccinated and exposed animals become carriers

# Negative marker cDNA-derived Killed FMDV Vaccine Platform



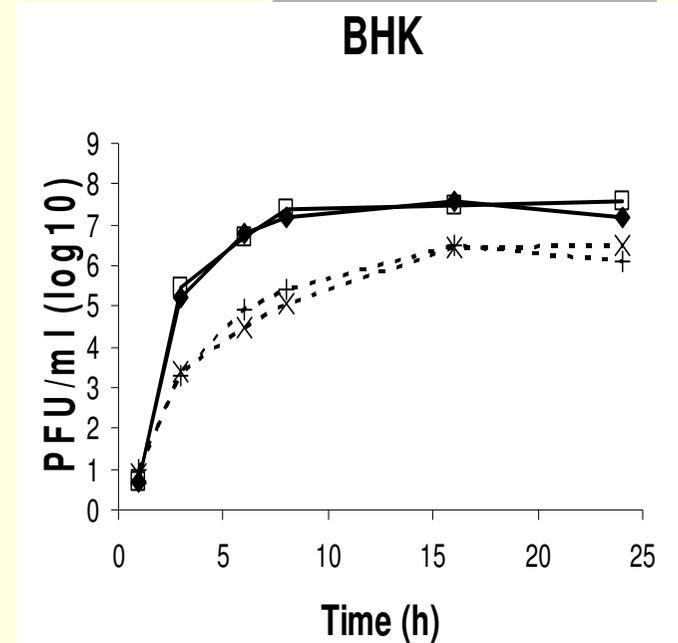
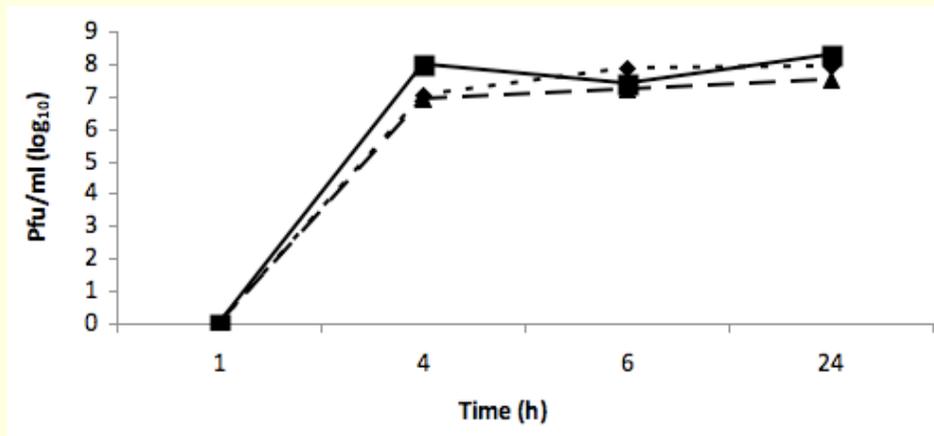
**Production of Vaccine antigens by Swapping  
Capsid sequences for those of Outbreak Strains**



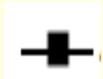


# *In vitro* Growth Characteristics

- Growth on BHK21 cell monolayers
- Titers 0.5-0.8 log lower than WT parental virus



- ◆— A<sub>24</sub> WT
- A<sub>24</sub> WT 3D<sub>m</sub>
- -×- - A<sub>24</sub> LL 3D<sub>m</sub>
- -+- - A<sub>24</sub> LL



A<sub>24</sub> WT



A<sub>24</sub> LL Asia1 P1 3B<sub>m</sub>3D<sub>m</sub>

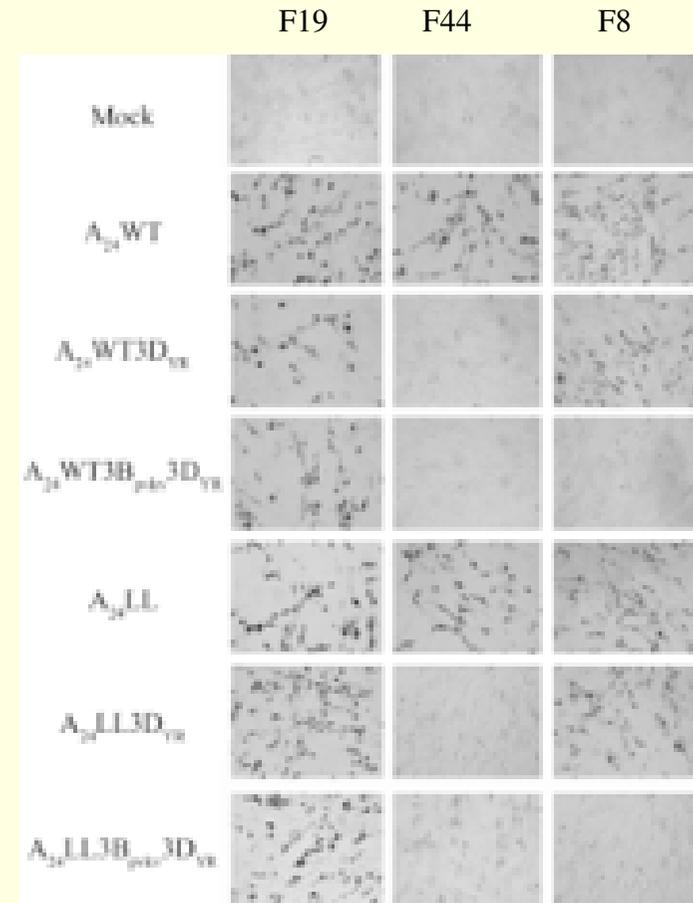


A<sub>24</sub> LL Turkey P1 3B<sub>m</sub>3D<sub>m</sub>

# Antigenic based distinction between A<sub>24</sub>LL 3B<sub>m</sub>3D<sub>m</sub> and parental viruses

## Characterization of Negative Marker FMD virus

Vaccine Source	Western Blot		
	anti3B-mAb	Anti 3D <sup>pol</sup> - mAbs	
		F8	F19
A <sub>24</sub> Cru (WT)	+	+	+
A <sub>24</sub> LL 3B <sub>m</sub> 3D <sub>m</sub>	-	+	-
A <sub>24</sub> LL Asia P1 3B <sub>m</sub> 3D <sub>m</sub>	-	+	-
A <sub>24</sub> LL Turkey P1 3B <sub>m</sub> 3D <sub>m</sub>	-	+	-



Two deleted epitopes and corresponding Mabs present opportunities for development of two DIVA diagnostic companion tests

# Genetic based distinction between A<sub>24</sub>LL 3B<sub>m</sub>3D<sub>m</sub> and parental viruses

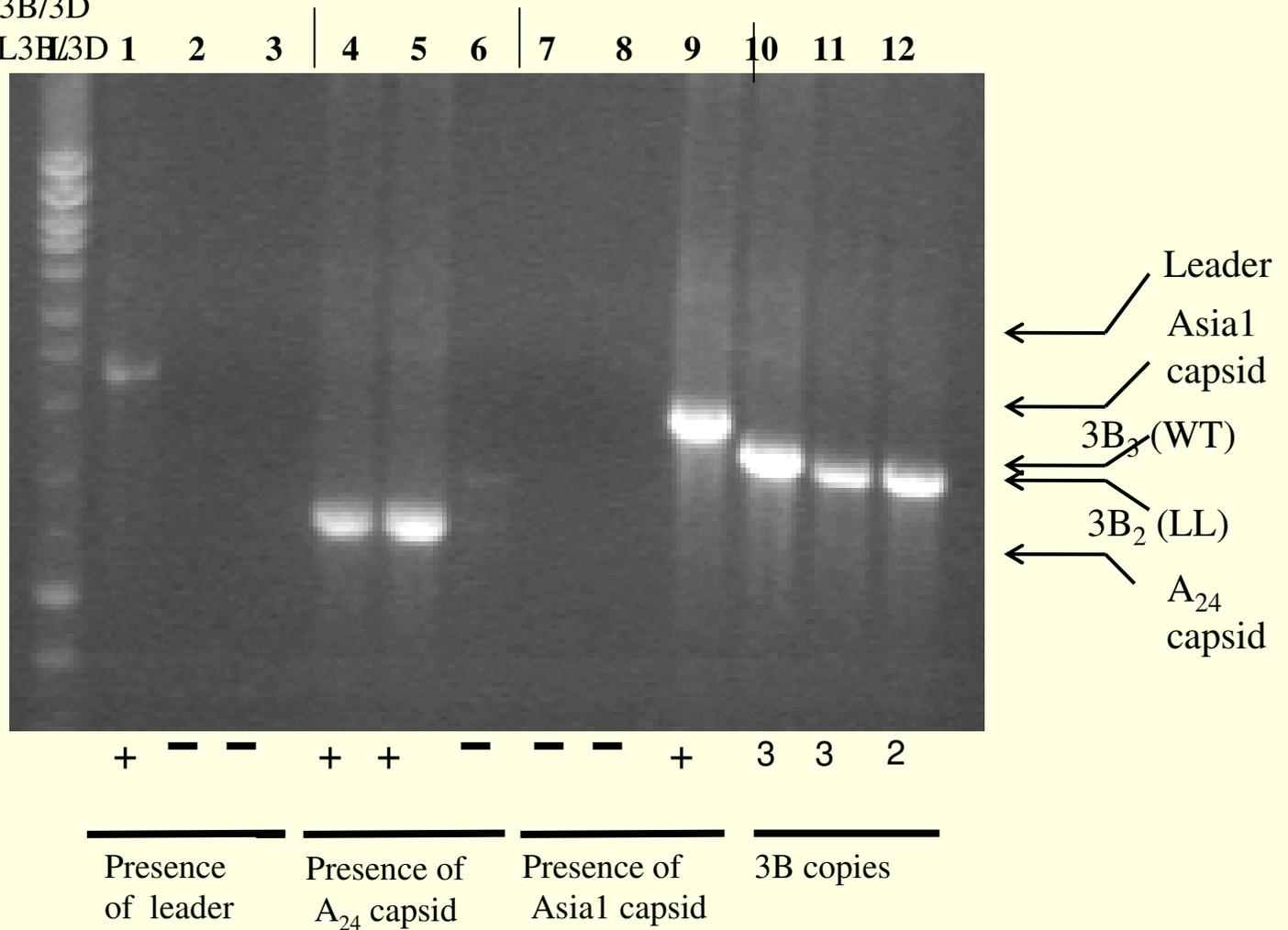
Lanes 1, 4, 7, 10: Wild type A<sub>24</sub> FMDV

Lanes 2, 5, 8, 11: A<sub>24</sub>FMDV LL3B/3D

Lanes 3, 6, 9, 12: Asia<sub>1</sub>FMDV LL3B/3D

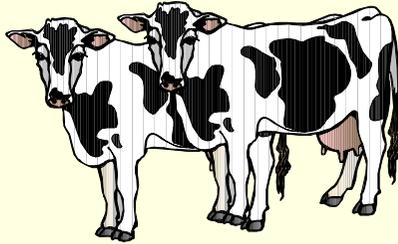
1. full genome sequence

2. RT-PCR screening

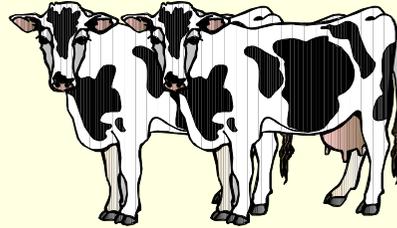


# Safety Data FMD-LL3B3D

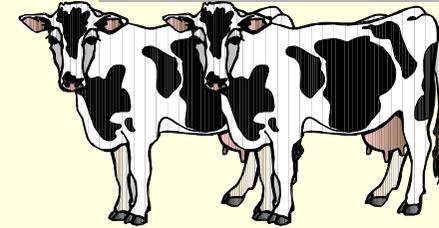
versus A<sub>24</sub>WT and 3B<sub>m</sub>3D<sub>m</sub> viruses in cattle



Bovines 7109, 7110  
A<sub>24</sub>WT



Bovines 9143, 9144  
A<sub>24</sub>WT 3B<sub>m</sub>3D<sub>m</sub>



Bovines 9145, 9146  
A<sub>24</sub>LL 3B<sub>m</sub>3D<sub>m</sub>

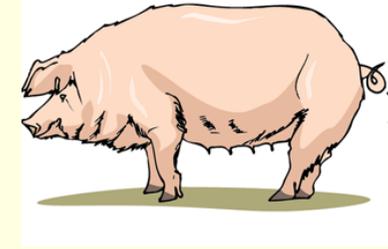
- Cows were inoculated by IDL (10<sup>6</sup> pfu live virus per cow)
- Clinical signs and temperatures were recorded for 10 days
- Samples collected for 10 days
  - Sera
  - Nasal swabs
  - Oral swabs
  - Room air samples
  - Vesicular fluid/tissue collected from lesions (if present)

# Safety Data Cattle

<b>Bovine #<sup>a</sup></b>	<b>Virus</b>	<b>Viremia, Maximum Titer.<sup>b</sup> (DPI)<sup>c</sup></b>	<b>Virus in Saliva, Maximum Titer<sup>b</sup> (DPI)<sup>c</sup></b>	<b>Fever<sup>d</sup> (DPI)<sup>e</sup></b>	<b>Maximum Clinical Score/ Maximum achievable<sup>f</sup> (DPI)<sup>g</sup></b>	<b>Neutralization Titer maximum (Starting DPI)<sup>h</sup></b>	<b>Shedding in air. Maximum Titer<sup>i</sup> (DPI)<sup>c</sup></b>
7109	<i>A<sub>24</sub>WT</i>	7.60 (3)	8.90 (3)	Yes (2,3)	5/5 (7)	2.4 (5)	5.57 (5)
7110	<i>A<sub>24</sub>WT</i>	7.31 (4)	10.18 (3)	Yes (2-5)	5/5 (5)	2.4 (6)	ND
9143	<i>A<sub>24</sub>WT3B<sub>m</sub>3D<sub>m</sub></i>	7.40 (4)	9.03 (5)	Yes (3)	1/5 (5)	3.6	6.29 (6)
9144	<i>A<sub>24</sub>WT3B<sub>m</sub>3D<sub>m</sub></i>	7.92 (4)	8.85 (5)	Yes (4)	4/5 (7)	3.0	5.45 (7)
9145	<i>A<sub>24</sub>LL3B<sub>m</sub>3D<sub>m</sub></i>	Negative	Negative	No	0/5	1.5	ND <sup>j</sup>
9146	<i>A<sub>24</sub>LL3B<sub>m</sub>3D<sub>m</sub></i>	Negative	Negative	No	0/5	2.4	Negative

FMD-LL3B3D is fully attenuated in cattle!

# Safety Data Pigs Determined with FMD-LL3D



Direct Inoculation (Pigs #40, #41) Direct Contact (Pigs#42; #43)

<b>Pig #40</b>	0 dpi <sup>a</sup>	1 dpi	2 dpi	3 dpi	4 dpi	5 dpi	6 dpi
Viremia <sup>b</sup>	Negative <sup>c</sup>	Negative	Negative	Negative	Negative	Negative	Negative
Virus in oral swab <sup>b</sup>	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Virus in nasal swab <sup>b</sup>	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Neutralization titer <sup>d</sup>	< 0.9	< 0.9	< 0.9	< 0.9	< 0.9	< 0.9	< 0.9
Clinical score	Negative	Negative	Negative	Negative	Negative	Negative	Negative
<b>Pig #41</b>	0 dpi	1 dpi	2 dpi	3 dpi	4 dpi	5 dpi	6 dpi
Viremia	Negative	5.44	Negative	Negative	Negative	Negative	Negative
Virus in oral swab	Negative	Negative	5.45	Negative	Negative	Negative	Negative
Virus in nasal swab	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Neutralization titer	< 0.9	< 0.9	< 0.9	< 0.9	0.9	1.2	1.2
Clinical score	Negative	Negative	Negative	Negative	Negative	Negative	Negative
<b>Pig #42</b>	0 dpc <sup>e</sup>	1 dpc	2 dpc	3 dpc	4 dpc	5 dpc	6 dpc
Viremia	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Virus in oral swab	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Virus in nasal swab	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Neutralization titer	< 0.9	< 0.9	< 0.9	< 0.9	< 0.9	< 0.9	< 0.9
Clinical score	Negative	Negative	Negative	Negative	Negative	Negative	Negative
<b>Pig #43</b>	0 dpc	1 dpc	2 dpc	3 dpc	4 dpc	5 dpc	6 dpc
Viremia	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Virus in oral swab	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Virus in nasal swab	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Neutralization titer	< 0.9	< 0.9	< 0.9	< 0.9	< 0.9	< 0.9	< 0.9
Clinical score	Negative	Negative	Negative	Negative	Negative	Negative	Negative

All room air samples negative

FMD-LL3D completely attenuated and non-transmissible in pigs!

# Safety Data in Pigs

Table 7. Responses of swine to infection with Asia1-A<sub>24</sub>LL3B3D or ATurkey06-A<sub>24</sub>LL3B3D viruses and virus shedding measurements.

Swine #	Virus	Inoculation route	Viremia, Maximum Titer. <sup>a</sup> (DPI) <sup>b</sup>	Virus in Saliva, Maximum Titer. <sup>a</sup> (DPI) <sup>b</sup>	Virus in Nasal Swab, Maximum Titer. <sup>a</sup> (DPI) <sup>b</sup>	Fever <sup>c</sup> (DPI)	Clinical Score	Neutralization Titer. <sup>d</sup> (DPI) <sup>e</sup>
199	Asia1/ A <sub>24</sub> LL3B <sub>PVKV</sub> 3D <sub>YR</sub>	Direct <sup>f</sup>	4.03 (2) <sup>h</sup>	Negative	Negative	No	Negative	1.5 (7)
200	Asia1/ A <sub>24</sub> LL3B <sub>PVKV</sub> 3D <sub>YR</sub>	Direct	Negative	Negative	Negative	No	Negative	<0.9
201	Asia1/ A <sub>24</sub> LL3B <sub>PVKV</sub> 3D <sub>YR</sub>	Direct	Negative	Negative	Negative	No	Negative	<0.9
197	Asia1/ A <sub>24</sub> LL3B <sub>PVKV</sub> 3D <sub>YR</sub>	Contact <sup>g</sup>	Negative	Negative	Negative	No	Negative	<0.9
198	Asia1/ A <sub>24</sub> LL3B <sub>PVKV</sub> 3D <sub>YR</sub>	Contact	Negative	Negative	Negative	No	Negative	<0.9
202	ATurkey06/A <sub>24</sub> LL3B <sub>PVKV</sub> 3D <sub>YR</sub>	Direct	Negative	Negative	Negative	No	Negative	1.8 (14)
203	ATurkey06/A <sub>24</sub> LL3B <sub>PVKV</sub> 3D <sub>YR</sub>	Direct	Negative	Negative	Negative	No	Negative	<0.9
204	ATurkey06/A <sub>24</sub> LL3B <sub>PVKV</sub> 3D <sub>YR</sub>	Direct	3.34 (1)	3.40 (7)	Negative	No	Negative	<0.9
205	ATurkey06/A <sub>24</sub> LL3B <sub>PVKV</sub> 3D <sub>YR</sub>	Contact	Negative	Negative	Negative	No	Negative	<0.9
206	ATurkey06/A <sub>24</sub> LL3B <sub>PVKV</sub> 3D <sub>YR</sub>	Contact	Negative	Negative	Negative	No	Negative	<0.9

<sup>a</sup> Expressed in log<sub>10</sub> RNA copy number/ml.

# Safety Data in Pigs

Table 8. Real time measurements of viral RNA on postmortem samples from swine inoculated with Asia1-A<sub>24</sub>LL3B3D (199 and 200) or ATurkey06-A<sub>24</sub>LL3B3D (203 and 204)

Tissue	Animal number			
	199	200	203	204
Inoculation site	NEG <sup>a</sup>	NEG	NEG	NEG
Tongue	NEG	NEG	NEG	NEG
Popliteal LNOD	NEG	NEG	2.36	NEG
Nasopharynx	NEG	NEG	NEG	2.34
Lung	NEG	NEG	NEG	NEG
Palatine tonsil	NEG	NEG	NEG	NEG
Coronary band	NEG	NEG	NEG	NEG

<sup>a</sup> Indicates FMDV RNA copy number per mg of tissue. Cutoff value is 2.26.

# Efficacy Data: Serological Response Cattle

Challenge



	Bovine #	Days post vaccination						
		0 <sup>(a)</sup>	7	14	21 <sup>(b)</sup>	28	35	42
FMDV tetraivalent Commercial Vaccine	863	<0.9 <sup>(c)</sup>	0.9	1.2	1.5	3.9	4.2	3.9
	864	<0.9	1.2	0.9	0.9	3.6	3.6	3.6
	865	<0.9	0.9	0.9	1.8	3.9	3.6	3.9
	866	<0.9	1.2	1.5	2.1	ND	ND	ND
A <sub>24</sub> LL3Dm vaccine	867	<0.9	2.1	2.4	2.7	3.3	3.3	3.0
	868	<0.9	2.4	2.1	2.7	3.3	3.6	3.9
	869	<0.9	2.1	2.4	2.7	3.0	2.7	3.0
	870	<0.9	2.1	1.8	2.4	3.0	3.3	3.0
A <sub>24</sub> LL3Bm3Dm vaccine	1018	<0.9	1.5	1.2	2.1	3.0	3.3	3.0
	1019	<0.9	1.8	1.5	2.1	3.3	3.6	3.0
	1020	<0.9	1.5	1.5	1.8	2.7	Euthanized*	-
	1021	<0.9	1.8	1.8	2.1	2.1	2.7	3.6
PBS (Controls)	871	<0.9	<0.9	<0.9	<0.9	2.4	3.0	3.6
	872	<0.9	<0.9	<0.9	<0.9	2.7	3.3	3.6
	1022	<0.9	<0.9	<0.9	<0.9	2.4	3.3	2.7
	1023	<0.9	<0.9	<0.9	<0.9	2.4	2.7	2.1

**Single Marker  
FMD vaccine**

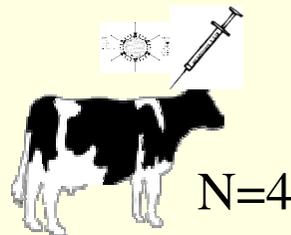
**Double Marker  
FMD vaccine**

- Euthanized due to unrelated health issue

**FMD-LL3D and FMD-LL3B3D induced high VN titers**

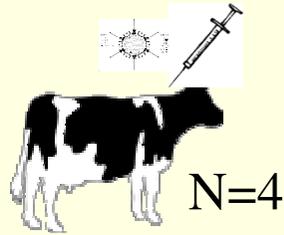
# Efficacy Data Cattle

BEI-inactivated Vaccine formulated with Montanide ISA 260 adjuvant (Seppic-WOW)



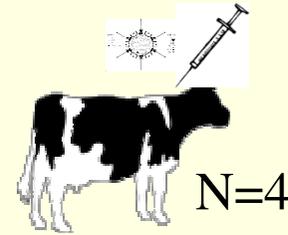
Commercial  
Tetravalent  
FMDV Vaccine  
1xBEI- Vx

No clinical disease  
(0/4)\*



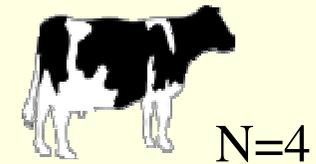
FMD-LL3D  
FMDV Vaccine  
1x BEI Marker virus

No clinical disease  
(0/4)\*



FMD-LL3B3D  
FMDV Vaccine  
1x BEI Marker virus

No clinical disease  
(0/4)\*



Naïve  
unvaccinated controls

100 % clinical  
disease (4/4)

Cattle were challenged by intradermolingual inoculation of 4 log 10 bovine infectious doses of FMDV A<sub>24</sub>WT.

Inactivated vaccines prepared with FMD-LL3D and FMD-LL3B3D induced complete protection against challenge!

# Efficacy Data A/Turkey06-LL3B3D

## Clinical score post challenge

Serum neutralization titers for bovine inoculated with BEI inactivated FMDV A/Turkey LL3B3D vaccine  
 Virus used for challenge: FMDV A/Iran

Bovine	DPV 0	DPV 7	DPV 14	DPV 21/DPC 0	DPV 28/DPC 7	DPV 35/DPC 14	DPV 42/DPC 21
BR-28	<0.9	1.2	1.2	2.4	3	3.3	3.3
BR-29	<0.9	2.4	2.1	1.8	2.4	3.9	3.3
BR-30	<0.9	2.4	1.5	1.2	2.4	3.3	2.4
BR-31	<0.9	1.5	<0.9	<0.9	3.3	3.6	3
BR-32	<0.9	<0.9	<0.9	<0.9	2.4	2.7	3.3
BR-33	<0.9	<0.9	<0.9	<0.9	2.4	2.7	3

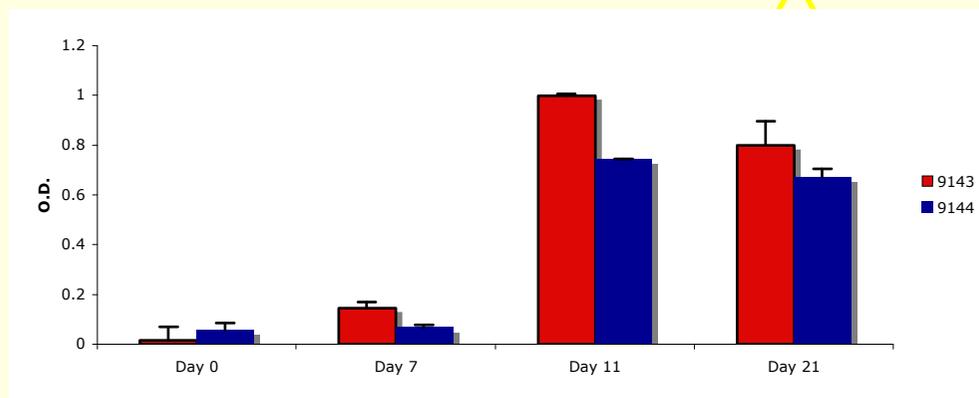
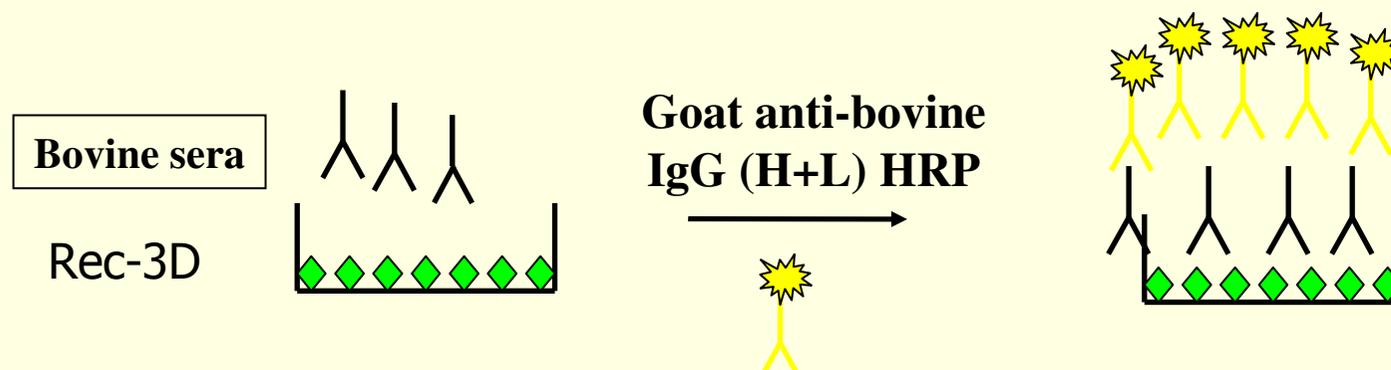
V100 titer 178 virus/well

Cow #	Treatment	DPI 0	DPI 3	DPI 7	DPI 10
28	A/Turkey06 Vaccinated	0	0	0	0
29	A/Turkey06 Vaccinated	0	0	0	0
30	A/Turkey06 Vaccinated	0	0	0	0
31	A/Turkey06 Vaccinated	0	0	1	1
32	Naïve Mock Vaccinated	0	1	2	3
33	Naïve Mock Vaccinated	0	4	4	4

Clinical score

# Assessment of 3-D Antibody Response by Indirect ELISA

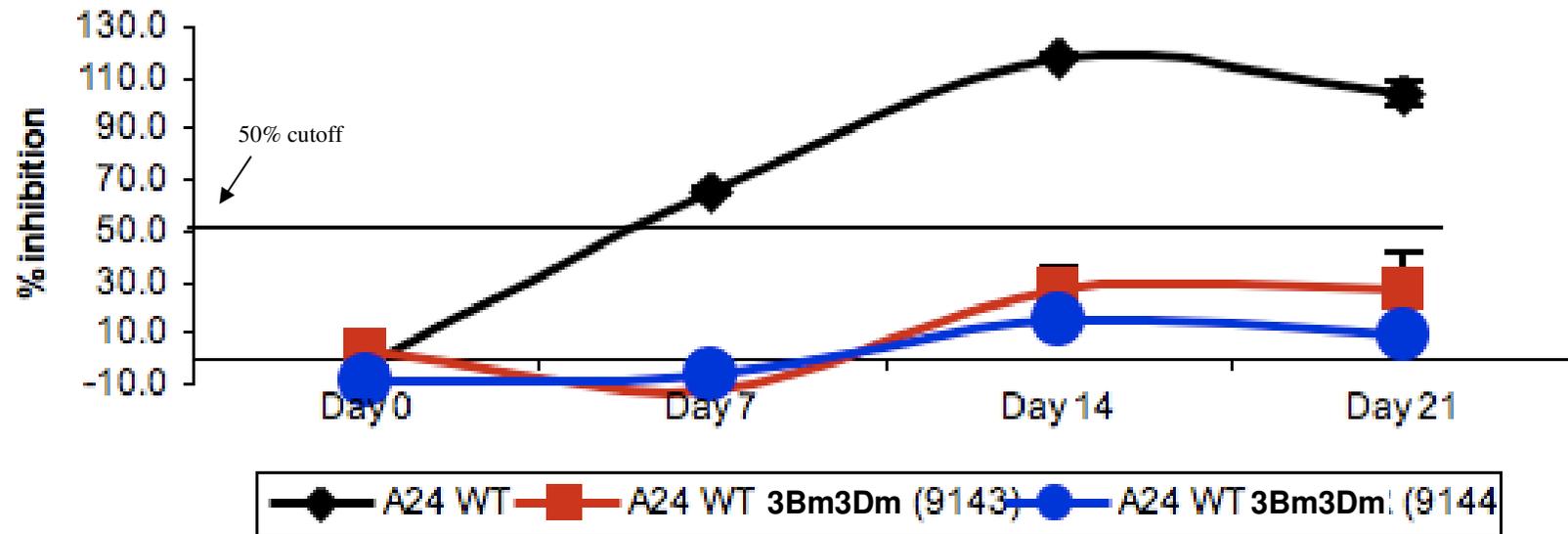
A<sub>24</sub>WT 3B<sub>m</sub>3D<sub>m</sub> Infected Bovines 9143-9144



Anti-3D antibodies

Animals Infected with FMD-wt3B3D make antibodies to 3D

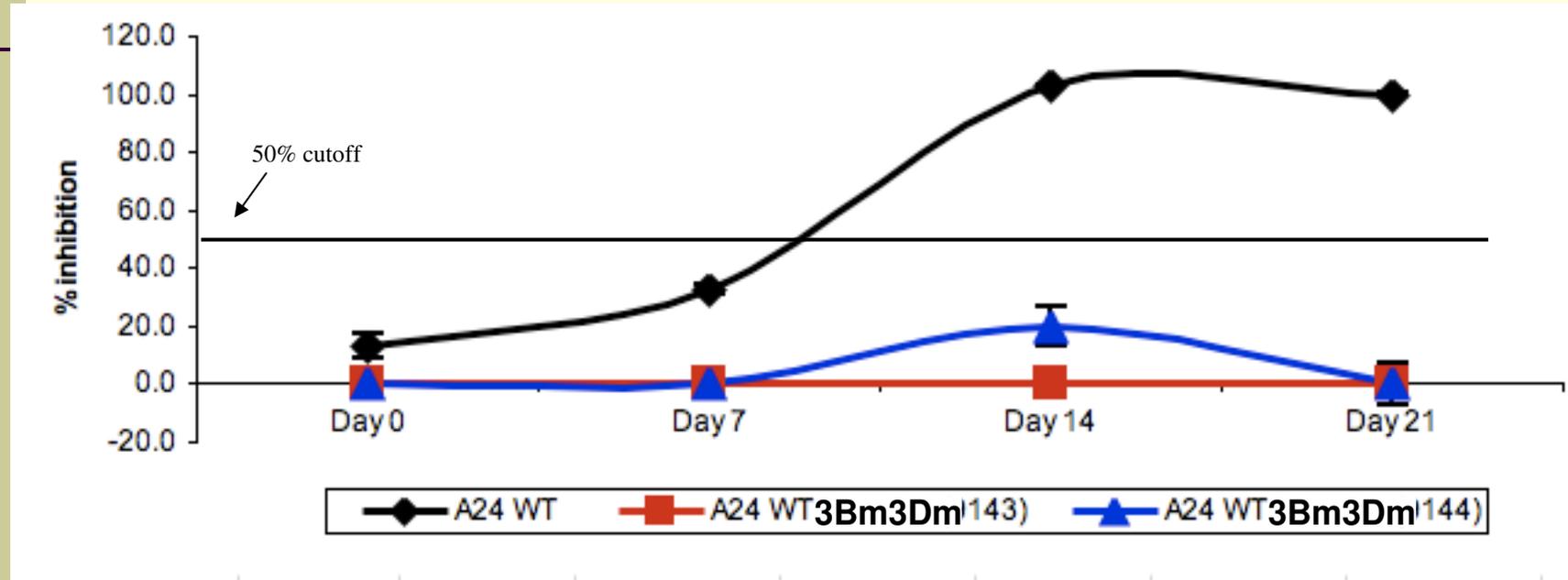
# Competitive DIVA 3D ELISA



Competing 3D Mab recognizes specific epitope deleted in 3D of FMD-LL3B3D

**DIVA-3D cELISA can differentiate between WT and FMD-LL3B3D Vaccinated Animals**

# Competitive DIVA 3B ELISA



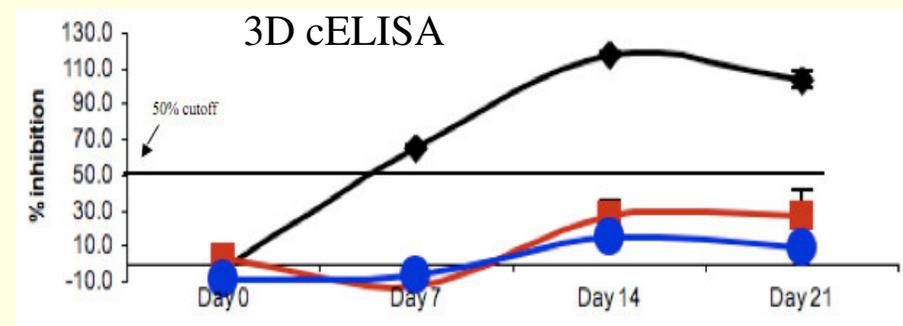
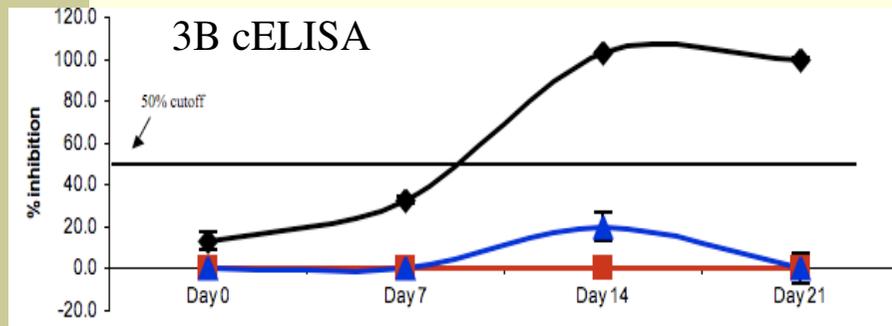
Competing 3B Mab recognizes specific 3B epitope deleted in FMD-LL3B3D

**DIVA-3B cELISA can differentiate between WT and FMD-LL3B3D  
Vaccinated Animals**

# Detection of antibodies against 3B and 3D<sup>pol</sup> in infected cattle

Indirect ELISA	Virus	Anti-3B antibodies	Anti-3D antibodies
	A <sub>24</sub> WT	+	+
A <sub>24</sub> WT 3B <sub>m</sub> 3D <sub>m</sub>	+	+	

## Competitive DIVA 3B and 3D<sup>pol</sup> ELISA



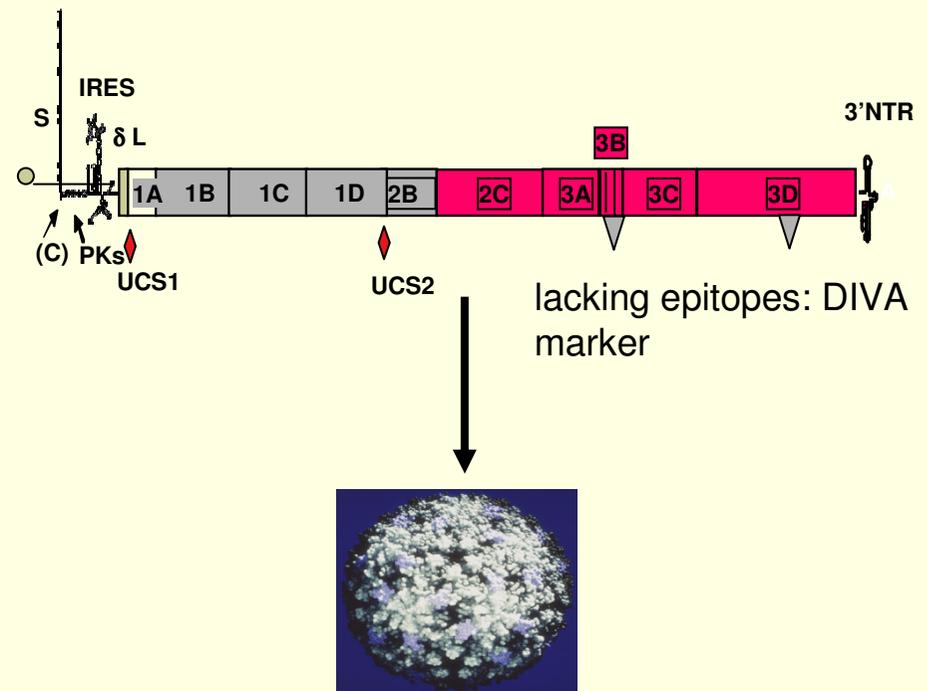
Competing 3B and 3D MAbs recognize specific epitopes that are absent in A<sub>24</sub> WT 3B<sub>m</sub>3D<sub>m</sub> virus

- ◆ A<sub>24</sub> WT
- A<sub>24</sub> WT 3B<sub>m</sub>3D<sub>m</sub> (9143)
- ▲ A<sub>24</sub> WT 3B<sub>m</sub>3D<sub>m</sub> (9144)

DIVA-3B and 3D cELISA can differentiate between WT and WT 3B<sub>m</sub>3D<sub>m</sub> infected animals

# Marker FMD-LL3B3D Vaccine Candidate

- **Safe production:** attenuated in cattle and pigs
- **Easy production:** uses same production system as current FMD vaccines
- **Simplified downstream processing:** no need for NSP removal
- **Non transmissible** from cattle and swine
- **Negative markers:** 2 independent DIVA compatible markers
- **Immunogenic:** same as current inactivated vaccine
- **Cassette construct** allows to rapidly insert capsid-coding region from emerging strains



# Acknowledgements

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