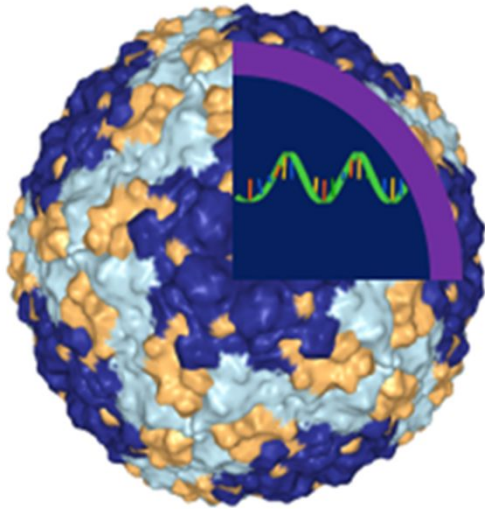


AN OVERVIEW OF 146S-SPECIFIC LLAMA SINGLE-DOMAIN ANTIBODY FRAGMENTS FOR USE IN FOOT-AND-MOUTH DISEASE VACCINE QUALITY CONTROL

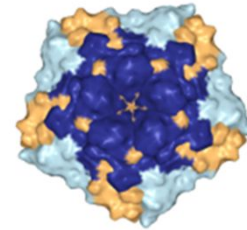
Michiel Harmsen



FMDV dissociation



Particle dissociation
during manufacturing
(heat, acidification)



146S

High immunogenic
Active ingredient

12S

Low immunogenic
Degradation product

Archiv für die gesamte Virusforschung 45, 362—364 (1974)
© by Springer-Verlag 1974

A Simple Method for the Quantification of 140S Particles of Foot-and-Mouth Disease Virus (FMDV)

Brief Report

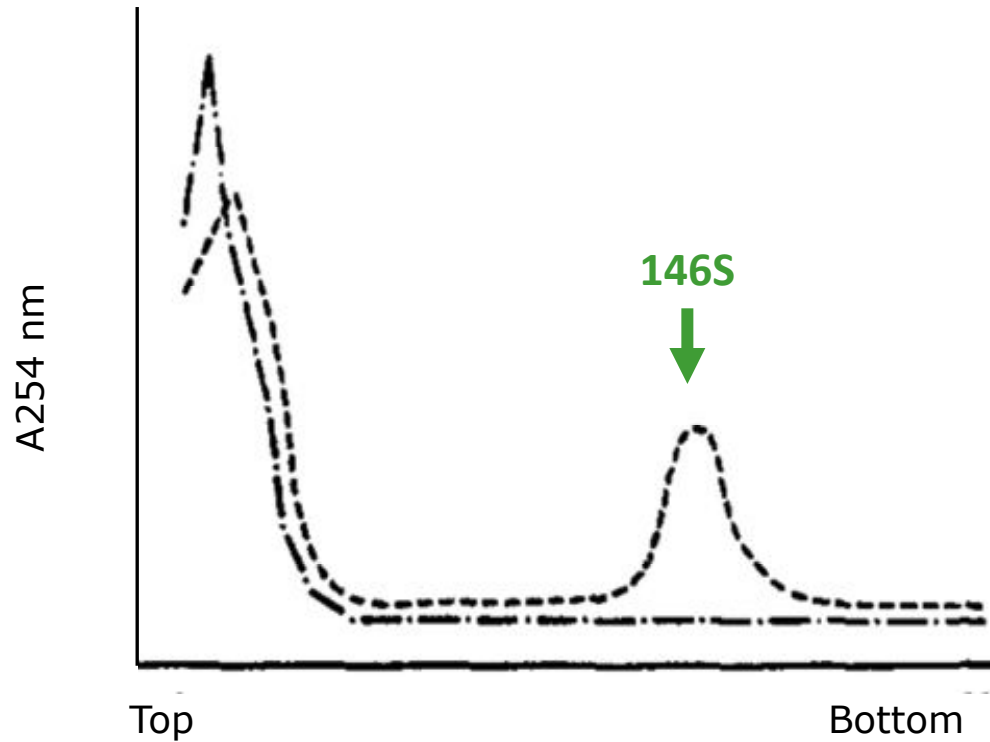
By

S. J. BARTELING and R. H. MELOEN

With 1 Figure

Received April 29, 1974

146S particle in Sucrose Density Gradients

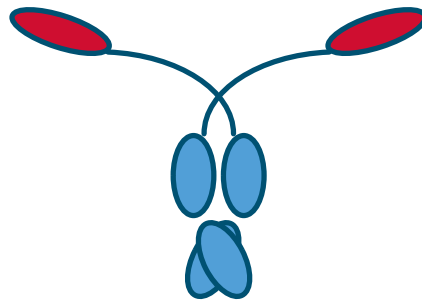


Single-domain antibody fragments (VHHs)

VHH



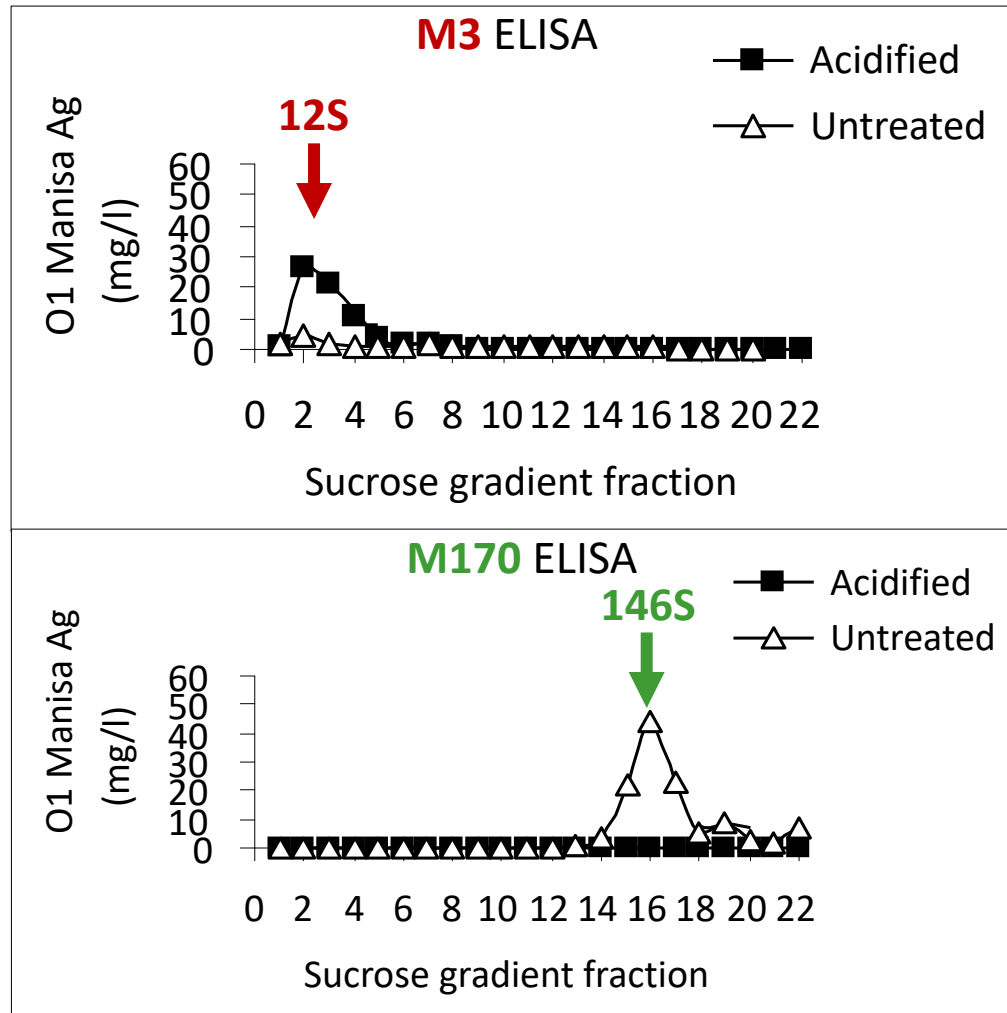
Heavy-chain



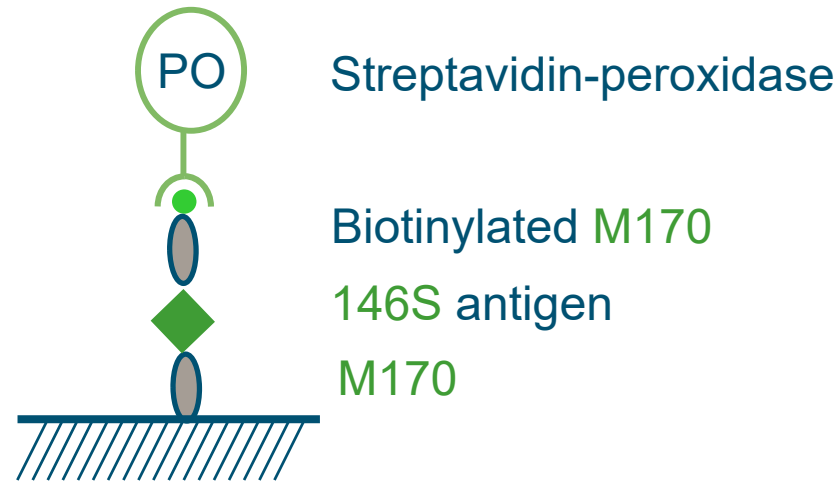
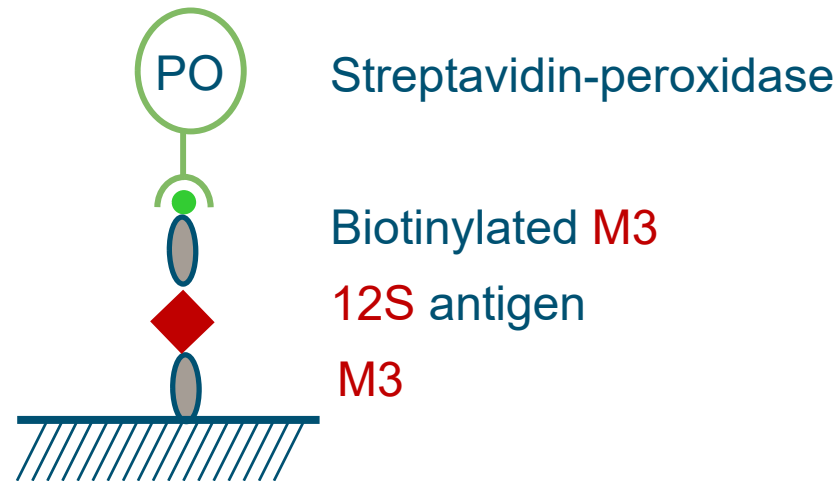
Advantages of VHH based ELISAs for 146S quantification

- Low limit of detection (~ 5 ng/ml)
- Higher throughput
 - 300 unknowns per day
- 146S specific VHHs are serotype and often strain specific:
 - Measurement in multivalent vaccines

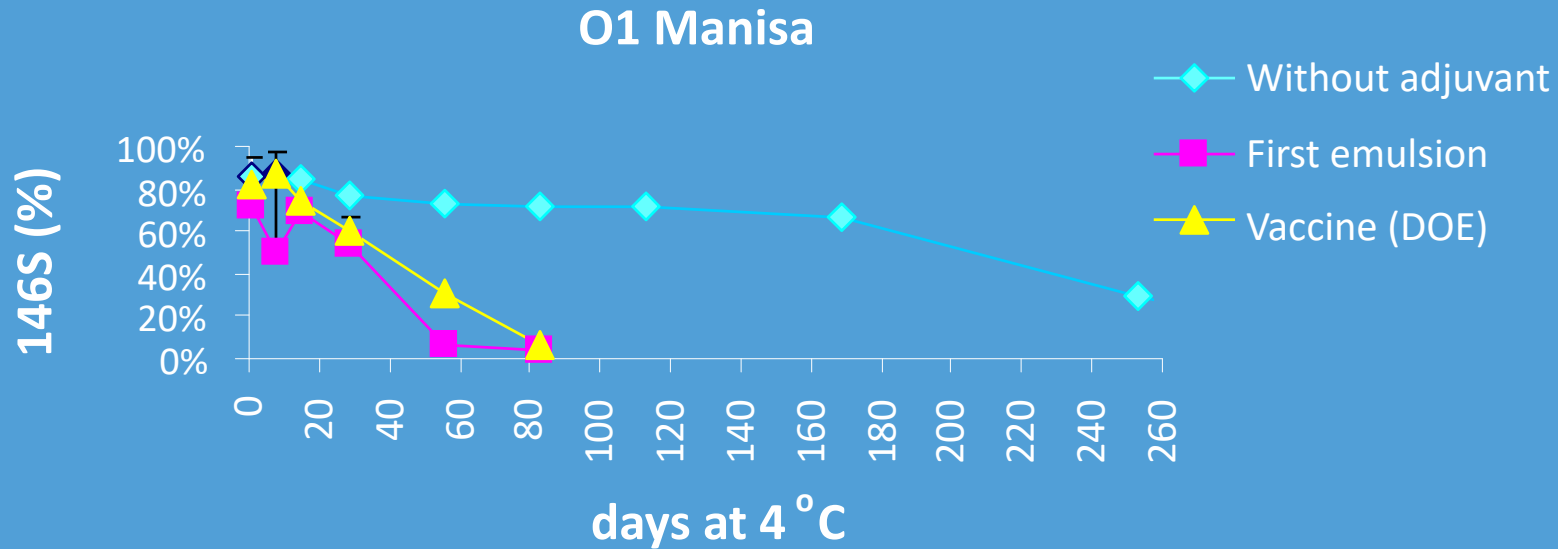
M3 binds 12S and M170 binds 146S



DAS ELISAs for 12S / 146S quantification



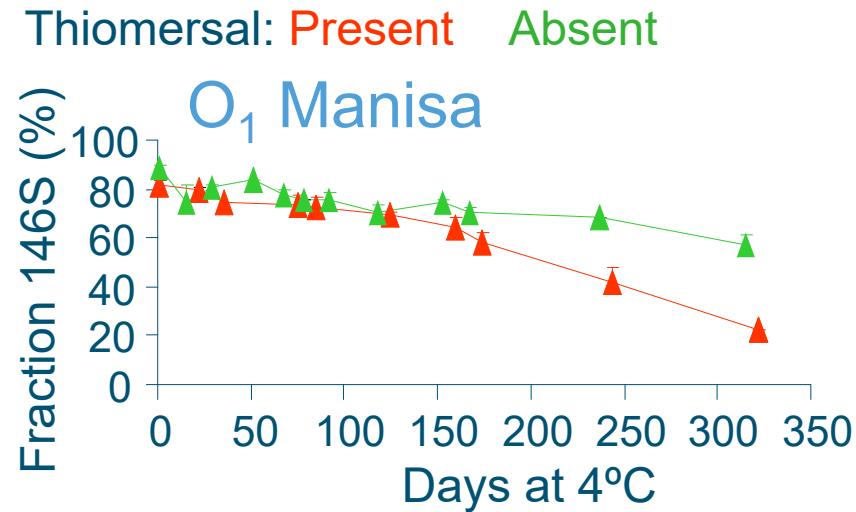
146S stability in oil-emulsion vaccine



➔ 146S dissociation due to presence of oil

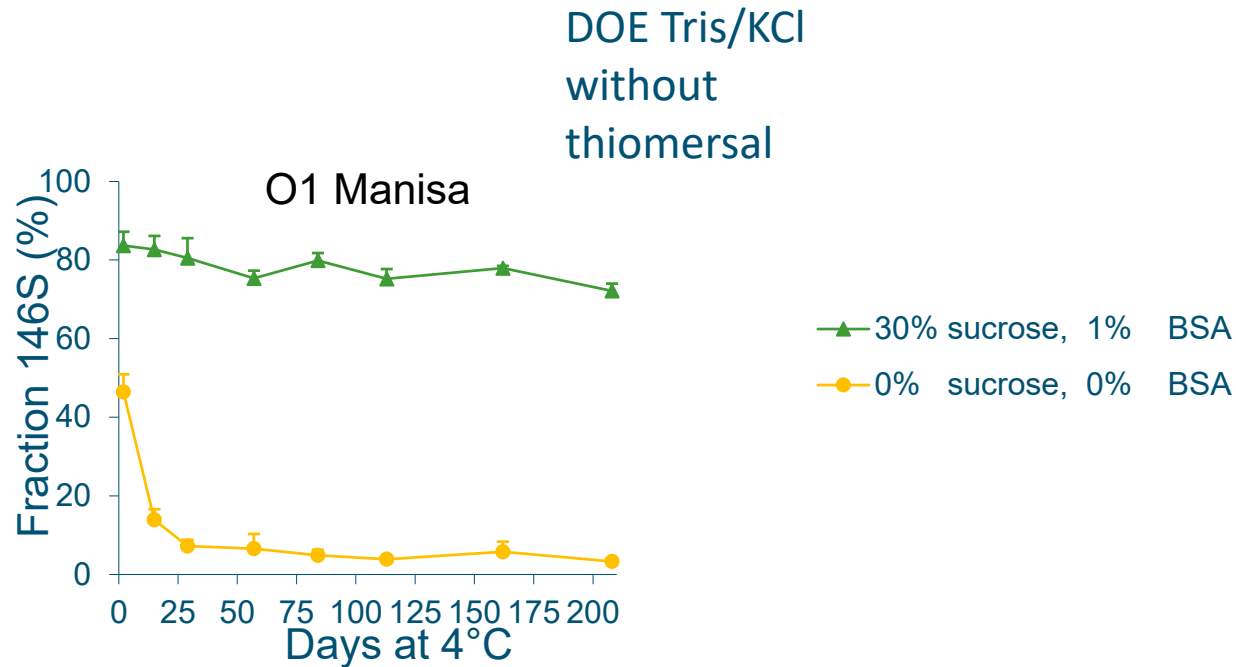
Harmsen et al. 2015, Vaccine 33, 2477-2484

FMDV stability +/- thiomersal at 4°C



Harmsen et al. 2011, Vaccine 29, 2682-2690

Effect BSA and sucrose on four strains



Harmsen et al. 2015, Vaccine 33, 2477-2484

Isolation of novel 146S specific VHHs against SAT2 and Asia 1 strains

Immunize llamas with SAT2 146S and Asia 1 Shamir 146S



Phage display selection on 146S
counterselection with 12S



146S specific VHHs



Yeast production of VHHs



M332F
Asia 1 Shamir



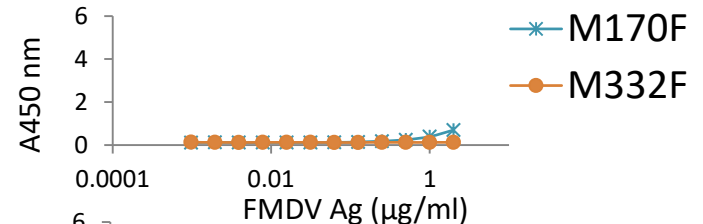
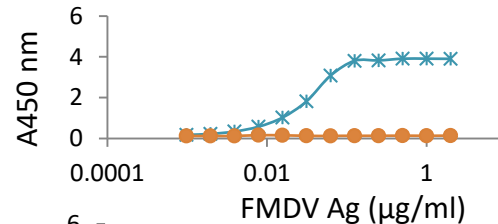
M377F
SAT2

146S specific VHHs are serotype specific

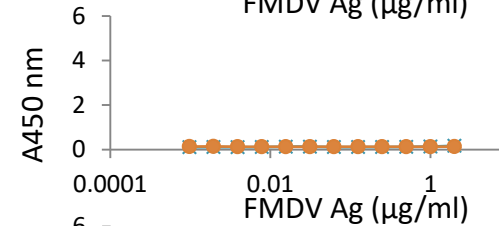
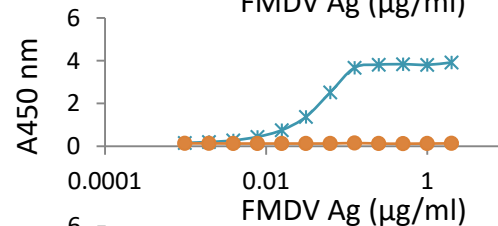
146S

12S

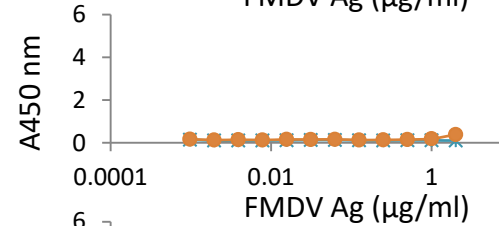
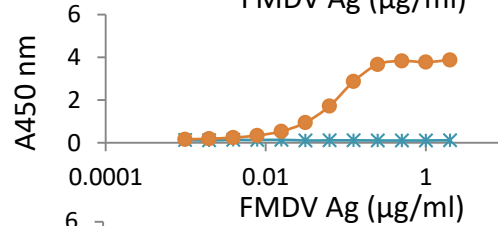
O1/Manisa/TUR/69



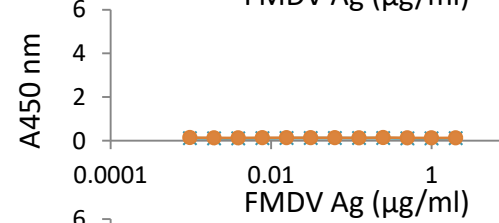
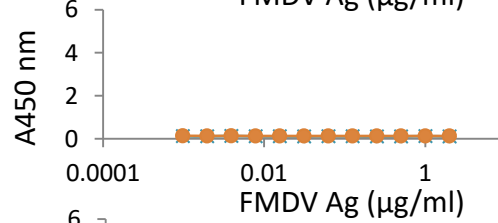
O1/BFS/UK/67



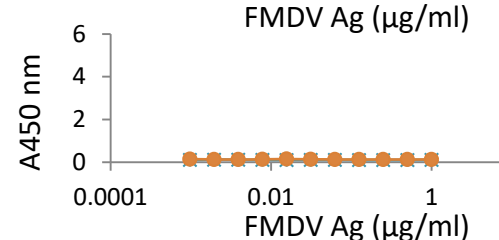
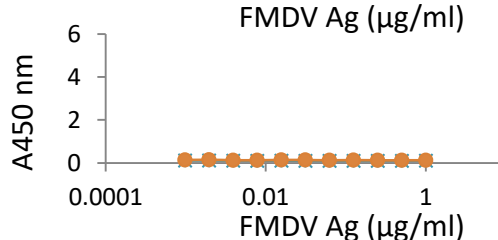
Asia1/SHA/ISR/89



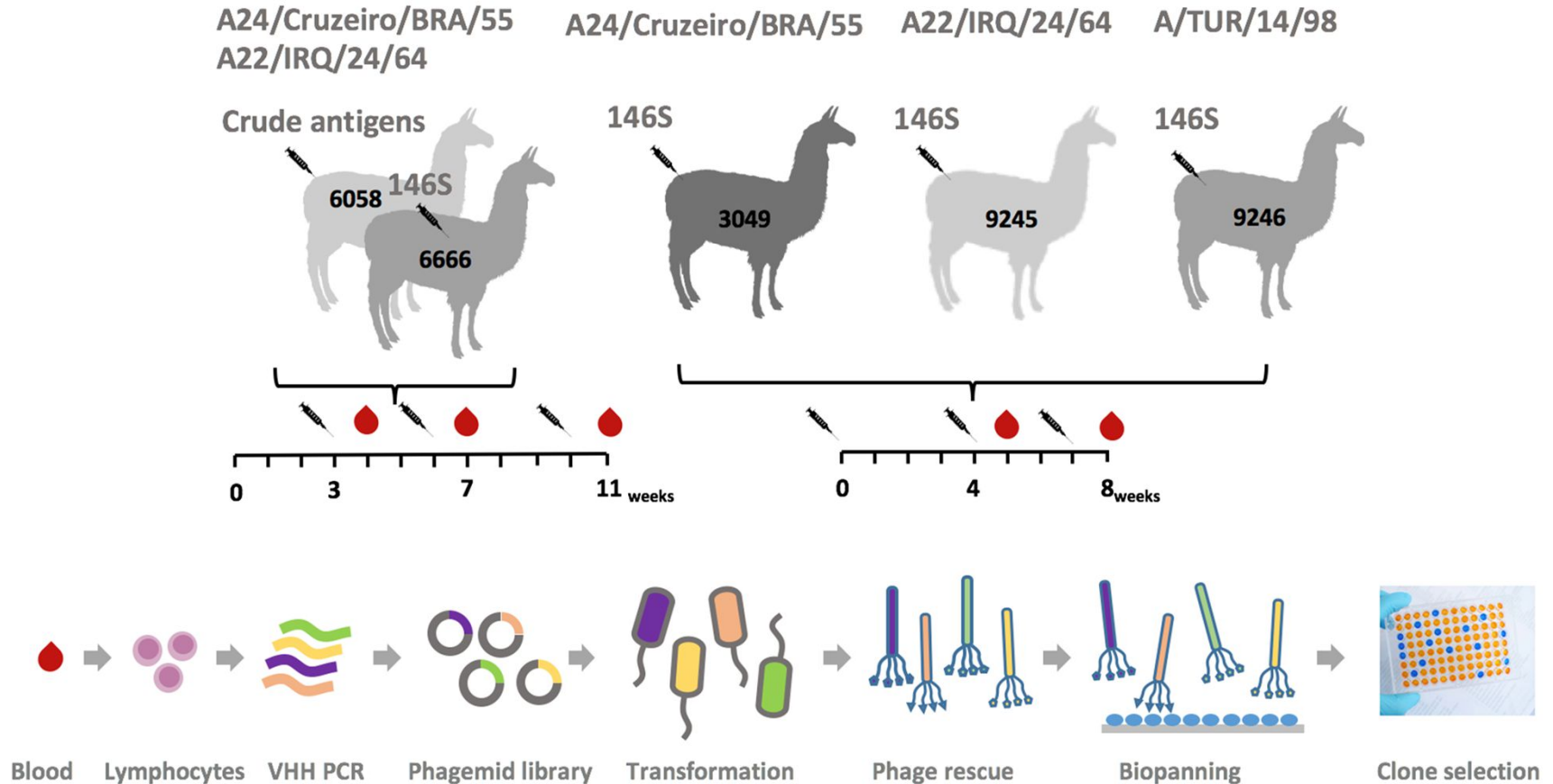
A24/CRU/BRA/55



A/TUR/14/98



Isolation of novel 146S specific VHs against serotype A



Among 24 novel VHHs 12 are 146S specific

VHH	CDR3 Group
M643F	Purple
M652F	
M659F	
M702F	
M676F	Red
M669F	
M686F	Orange
M688F	
M677F	Black
M678F	Blue
M691F	Green
M703F	

Novel 146S-specific llama VHHs for use in FMD serotype A vaccine quality control



Li, Haozhou^{1,2}; Harmsen, Michiel M³; Dekker, Aldo³; Sun, Shiqi² & Kortekaas, Jeroen^{1,3}



¹Laboratory of Virology, Wageningen University, Wageningen, the Netherlands
²State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, China
³Wageningen Bioveterinary Research, Lelystad, the Netherlands

Background

FMD vaccine efficacy is largely determined by the amount of intact (146S) virions which are most potent in inducing neutralizing antibodies. Dissociation of 146S particles or particles lacking the RNA (75S) into 12S particles (Fig. 1A) strongly reduces vaccine potency. Several FMDV 146S-specific llama single-domain antibody fragments (VHHs) have been earlier isolated against serotypes O, SAT2 and Asia 1 Shamir for use in double antibody sandwich (DAS) ELISAs to quantify the 146S content during vaccine manufacturing, formulation and storage. However, serotype A 146S-specific VHHs are still lacking.

Objective

Isolate 146S specific VHHs that recognize a broad panel of serotype A strains for vaccine quality control.

Method

Five llamas were immunized with crude or purified 146S FMDV antigens (Fig. 1B). Isolation of 146S specific VHHs was done by two rounds of phage display selection from llama immune libraries (Fig. 1C). Then clones were sequenced and grouped by their third complementarity-determining region (CDR3). Isolated VHHs were

FMDV strain	Absorbance values in ELISA of 146S or 12S of various FMDV strains											
	VHH1	VHH2	VHH3	VHH4	VHH5	VHH6	VHH7	VHH8	VHH9	VHH10	VHH11	VHH12
146S	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
12S	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Figure 2. Binding of twelve 146S specific VHHs to 12S and 146S of various FMDV strains of different serotypes in ELISA. A total of thirteen FMDV serotype A strains and other C, O, Asia1 strains were used. VHHs with similar CDR3s are grouped by color (black represents unique CDR3s). The order of FMDV strains from left to right is arranged according to their phylogenetic relationship (Fig. 3).

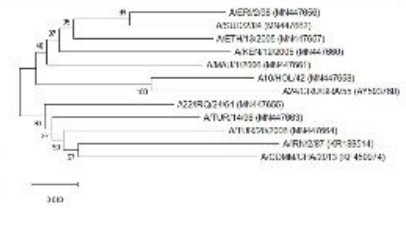
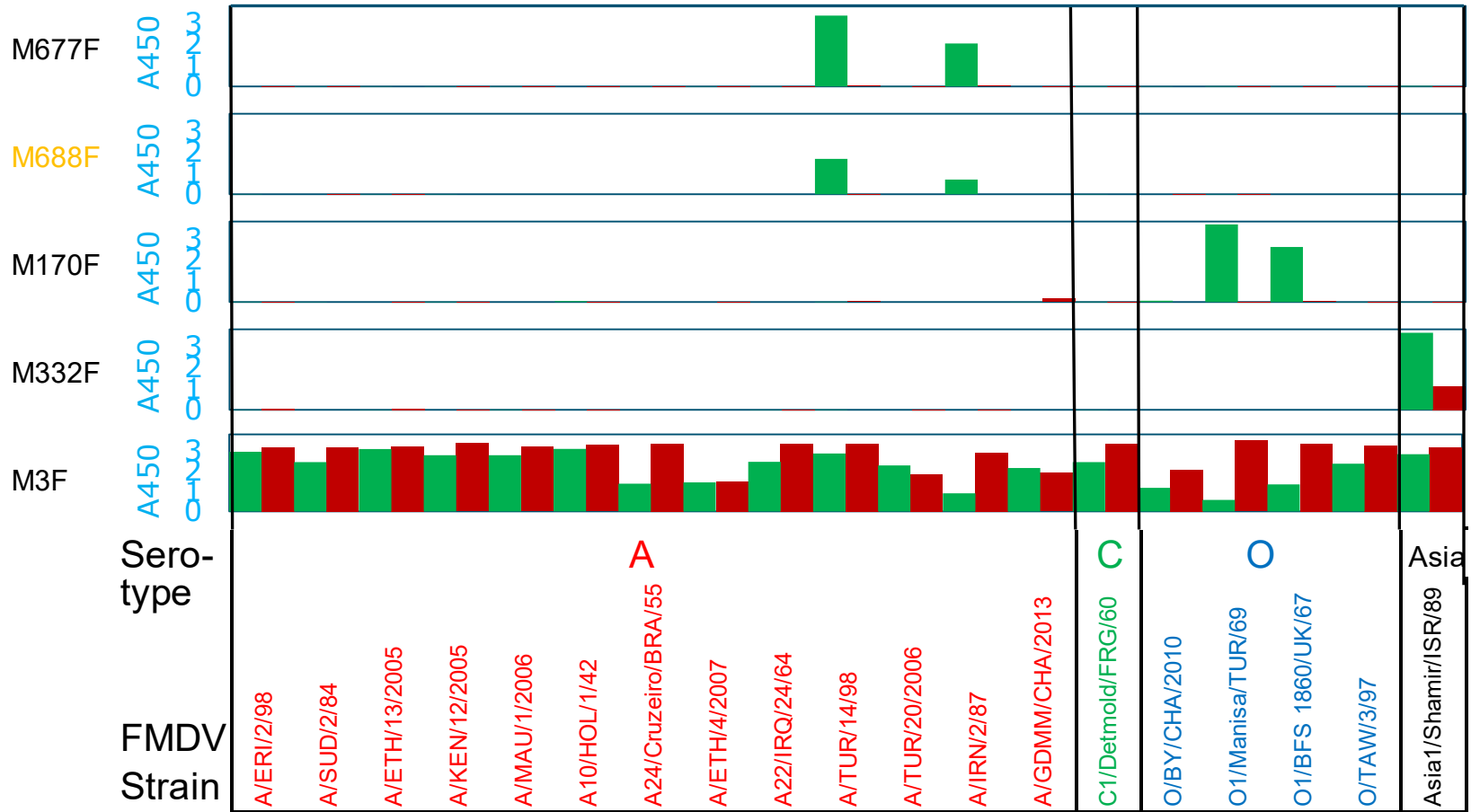


Figure 3. Unrooted neighbor-joining tree showing the phylogenetic relationships between the FMDV serotype A strains. P1 protein sequences of database accession numbers indicated after strain names were used. The evolutionary distances were computed using the Poisson correction model and 500 replicates for bootstrap in MEGA-X.

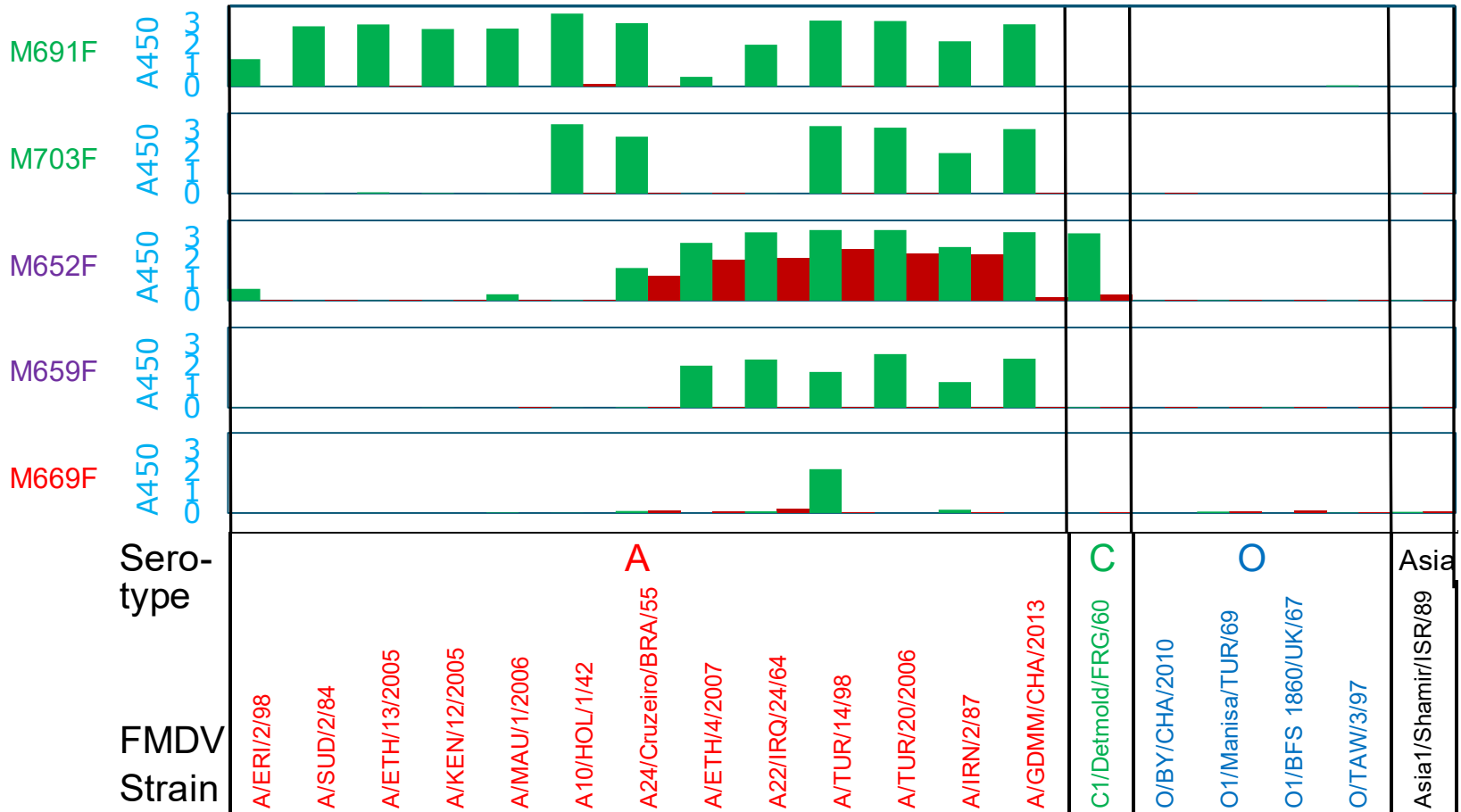
However, the 4 VHHs from the purple CDR3 group bind at most 7 out of 13 serotype A strains that are also more closely related phylogenetically (Fig. 3). Importantly, M691F binds to all 13 serotype A strains

VHH	Comp. no.	VHH	By inhibition of binding of biotinylated VHH														
			VHH1	VHH2	VHH3	VHH4	VHH5	VHH6	VHH7	VHH8	VHH9	VHH10	VHH11	VHH12			
M691F	13	Yes	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
M691F	13	No	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

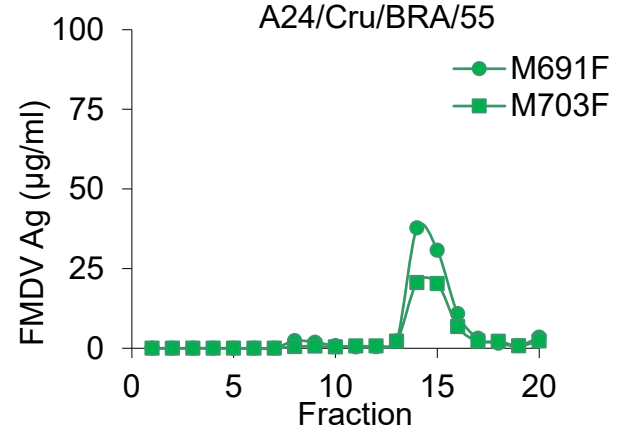
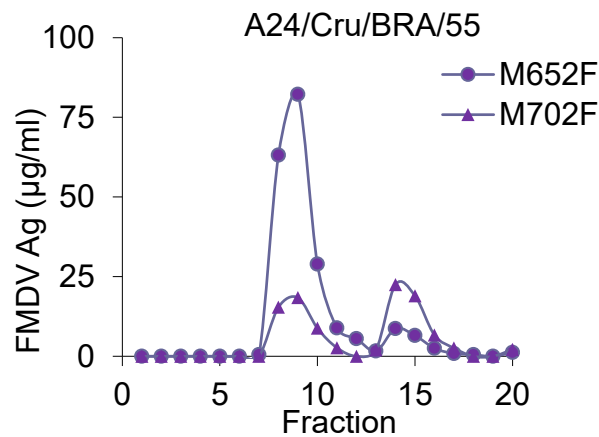
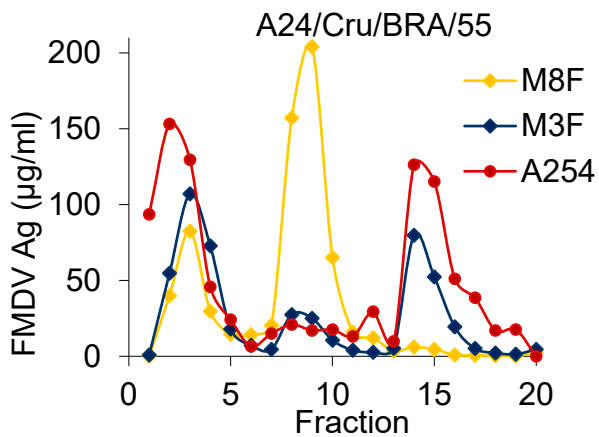
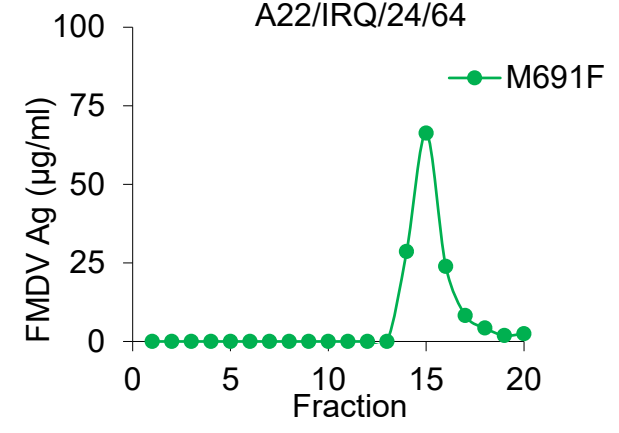
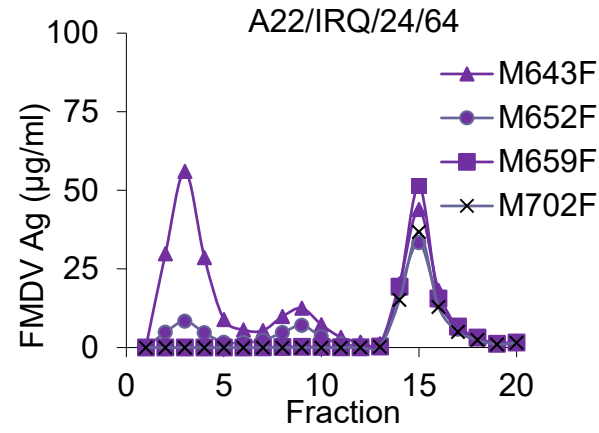
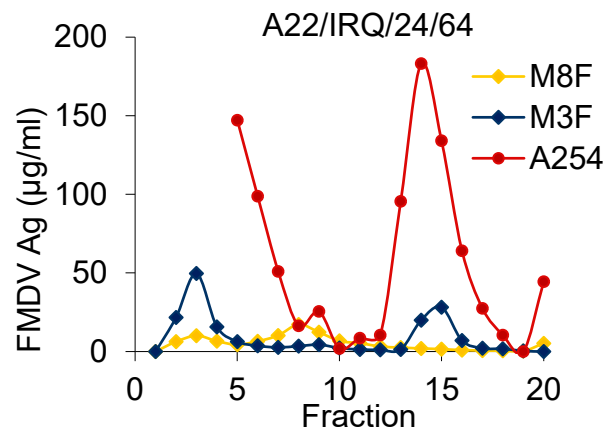
Novel 146S specific VHHs for serotype A (1)



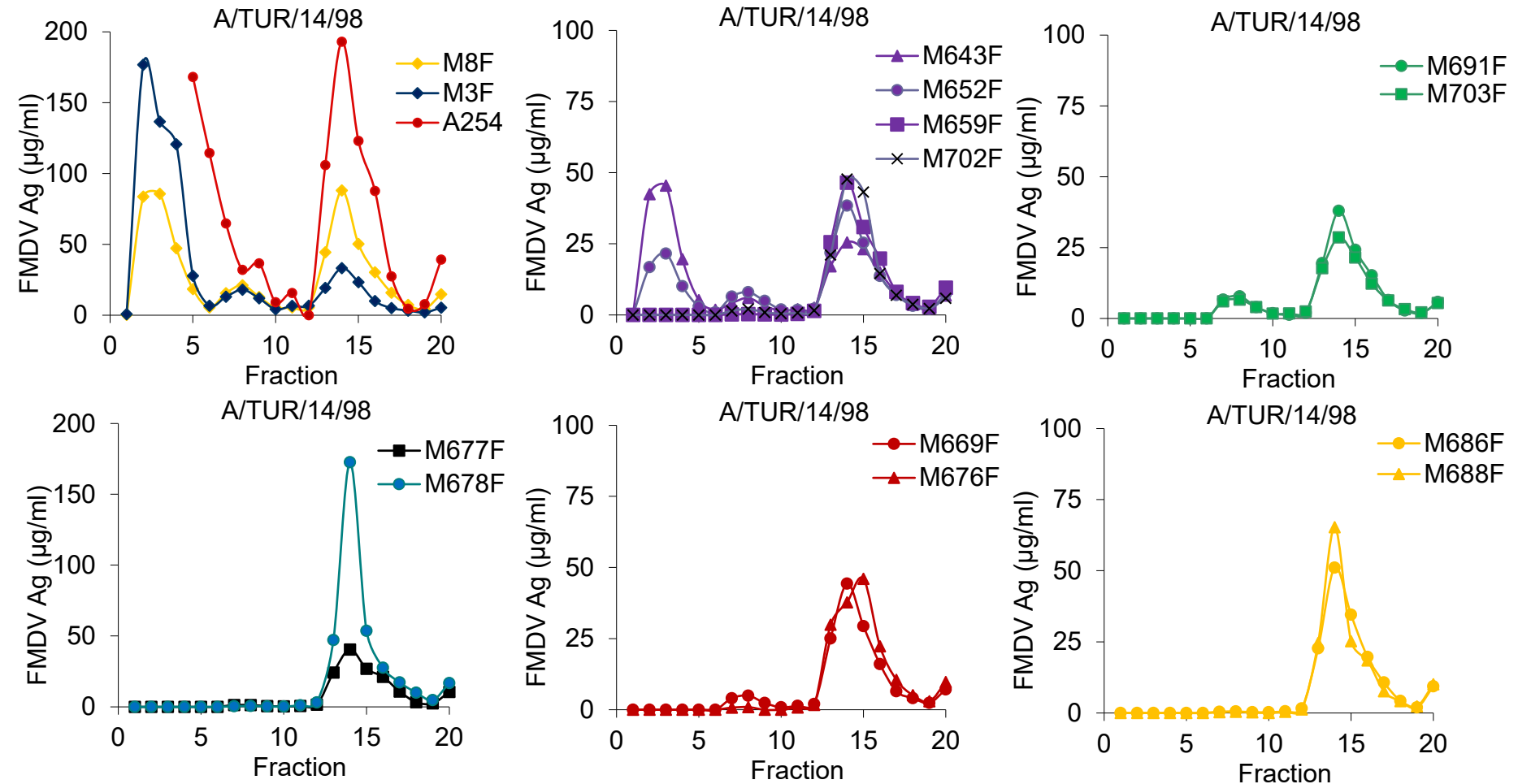
Novel 146S specific VHHs for serotype A (2)



Binding in ELISA to SDG fractionated FMDV



Binding in ELISA to SDG fractionated FMDV



Competition ELISA shows two sites

% inhibition of binding of biotinylated VHH

	M3F	M663F	M680F	M665F	M684F	M675F	M220F	M643F	M652F	M659F	M702F	M691F	M703F	M669F	M676F	M677F	M678F	M661F	M651F	M8F	M686F	M688F	M679F
M3F	94	14	74	-13	19	1	6	4	3	0	6	-2	19	-13	-4	0	-8	13	4	4	-2	1	-7
M663F	102	90	100	4	36	10	13	-4	0	-10	4	7	30	-5	-2	0	0	8	6	4	5	8	-6
M680F	99	66	96	17	4	17	2	1	8	9	23	-7	6	-6	-1	4	0	-6	-5	1	-13	-6	-10
M665F	110	104	104	98	88	49	13	-26	20	6	-38	1	21	18	41	29	42	-53	-7	-40	22	23	18
M684F	101	99	101	64	86	73	23	-3	-3	-3	-14	-13	-12	-1	17	7	11	25	24	13	12	14	21
M675F	8	1	10	5	6	86	1	8	8	-3	6	4	13	-3	-3	9	0	25	2	-3	5	-1	9
M220F	-26	-29	-12	-6	17	27	93	-3	-26	-7	-33	-31	-8	5	2	3	4	18	-3	-22	10	10	4
M643F	7	2	1	8	2	9	6	87	85	24	36	42	42	23	18	34	30	6	32	0	10	12	14
M652F	4	-3	1	0	0	8	-3	72	95	44	58	62	57	52	29	58	46	18	12	24	6	3	8
M659F	3	7	-11	14	-1	-2	8	98	100	94	100	101	100	99	82	100	97	60	59	64	24	33	15
M702F	0	1	-1	-1	2	-1	-3	86	98	74	95	98	96	90	60	95	76	18	49	67	7	11	3
M691F	-4	-5	11	-2	-1	10	-1	42	79	25	63	98	96	82	31	48	21	87	14	-5	3	12	9
M703F	11	16	13	4	9	13	17	80	96	70	93	101	100	98	68	97	86	98	17	49	25	30	12
M669F	-5	-1	-15	2	4	-7	-8	56	84	44	72	100	99	95	50	96	84	92	-1	12	13	15	4
M676F	9	15	-19	7	9	-21	14	95	98	94	97	100	99	100	97	100	92	100	21	81	49	60	30
M677F	-6	-1	-3	9	3	2	1	84	98	74	90	99	100	98	80	99	89	97	5	14	15	30	3
M678F	-8	-4	-6	8	6	1	-7	80	96	67	93	101	100	100	37	101	93	94	17	9	14	19	-1
M661F	4	4	-2	6	2	7	3	2	5	5	6	10	16	8	8	14	-5	94	8	-2	0	-1	5
M651F	10	2	-8	2	-4	6	3	36	30	13	44	3	-8	0	-8	4	18	93	76	51	6	2	1
M8F	5	-3	8	-9	11	2	3	5	-7	5	4	0	3	-9	4	-9	4	4	4	94	5	4	29
M686F	5	1	-11	16	10	16	-8	28	22	26	22	7	12	21	21	41	23	50	22	90	94	93	98
M688F	13	8	-9	13	2	5	0	26	22	18	24	12	12	21	18	40	26	45	29	88	89	91	95
M679F	9	-1	-6	13	3	1	1	4	6	7	9	7	-4	5	7	8	6	10	4	69	41	45	87

Site 1

Site 2

Conclusions novel 146S specific VHHs

- 146S specific VHHs with broader strain recognition do exist
 - Some 146S specific VHHs even bind 2 serotypes
- 146S specificity varies between
 - FMDV strains
 - VHHs from same CDR3 group
- Antigenicity of 75S and 146S differs
- Two 146S specific antigenic sites exist

Overview of 146S specific VHHs

VHH	146S Specificity	FMDV Strains
M170F	High	O1/Manisa/TUR/69, O1/BFS/67
M210F	High	O1/Manisa/TUR/69, O1/BFS/67
M332F	High	Asia 1/Sha/ISR/89
M658F	High	Asia 1/Sha/ISR/89
M377F	High	SAT2 SAU/2/00
M378F	High	SAT2 SAU/2/00
M379F	High	SAT2 SAU/2/00
M652F	Low	9 out of 13 serotype A strains and C1 Detmold
M659F	High	7 out of 13 serotype A strains
M691F	High	13 out of 13 serotype A strains

Thank you for your attention!
