



Development of  
rapid detection lateral flow strip kit  
for Foot-and-Mouth Disease Virus serotype O,A  
and Asia1 in clinical samples

**Bok Kyung Ku**, Jin-ju Naa, Soyeon Ryoo, Sang Hyun  
Park, Woo Jin Lee, Jong-Hyeon Park, Jae-Won Byun, Moon  
Kyun Shin, Mi-Young Park, Hyun Mi Pyo, Bong-Kyun Park

FMD Division, Animal and Plant Quarantine Agency, Republic of Korea

# Introduction

## ▪ Situation

- There have been a total nine outbreaks in Korea.
- FMD and occurred every year since 2014.
- For the first time, two serotypes (O and A) of FMD occurred simultaneously in 2017.

## ▪ FMD SOP in Korea

- In case of FMD occurrence with non-vaccinated serotypes( → Emergency situation)

## ▪ Interested in

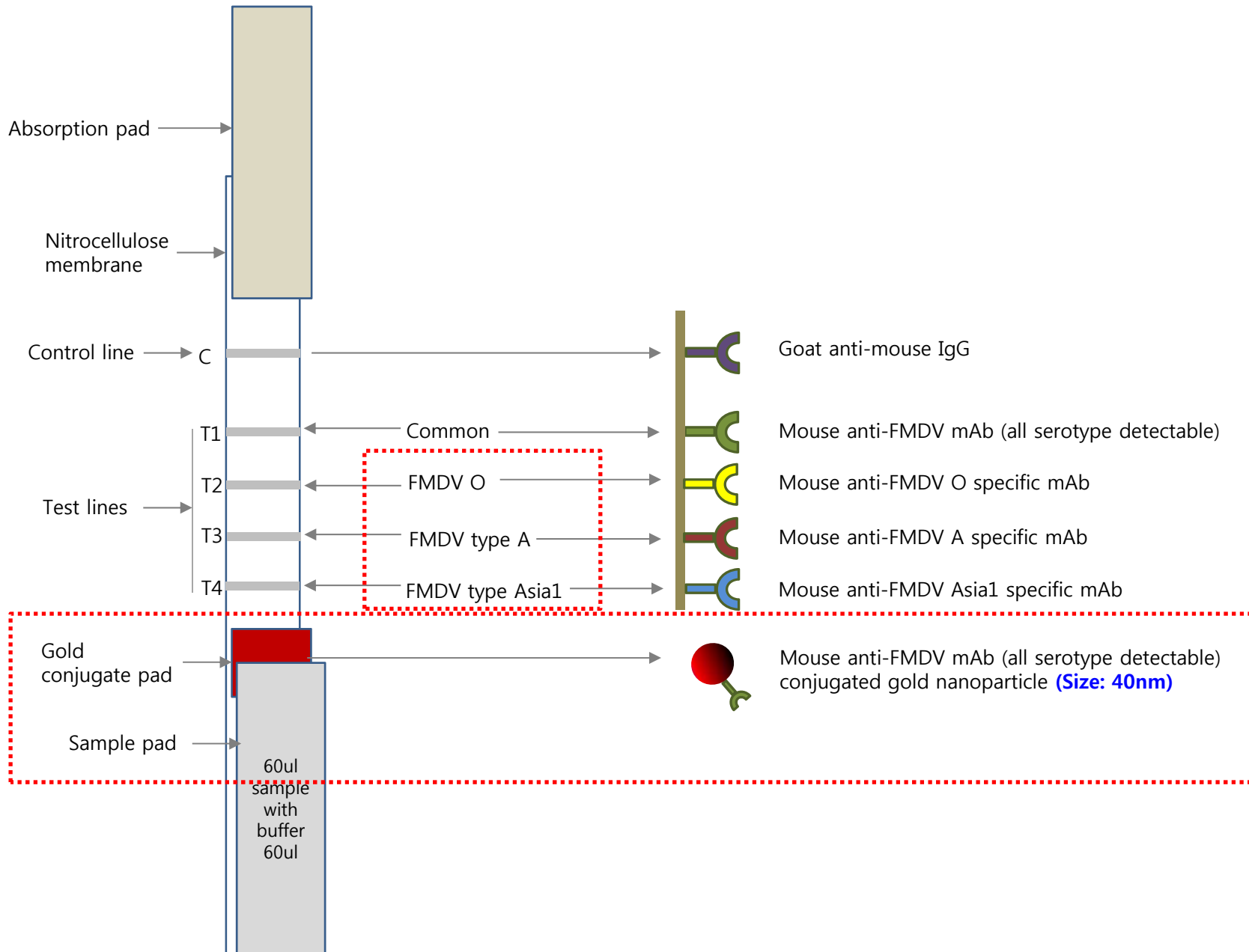
- A rapid and easy tool which can distinguish the serotype of FMDV for the on-site diagnosis of suspected FMD outbreaks.

## ▪ AIM

- To develop a lateral flow strip test for rapid detection of FMD virus serotypes O, A and Asia1 which mainly found in near country, East Asia.

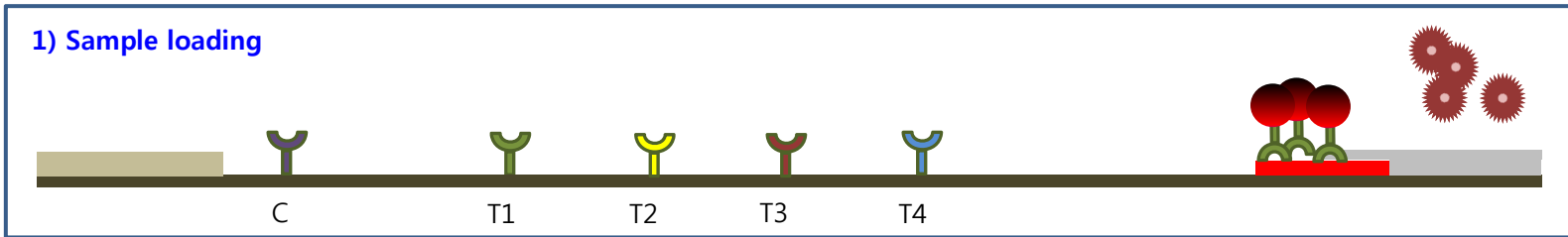


# Schematic diagram

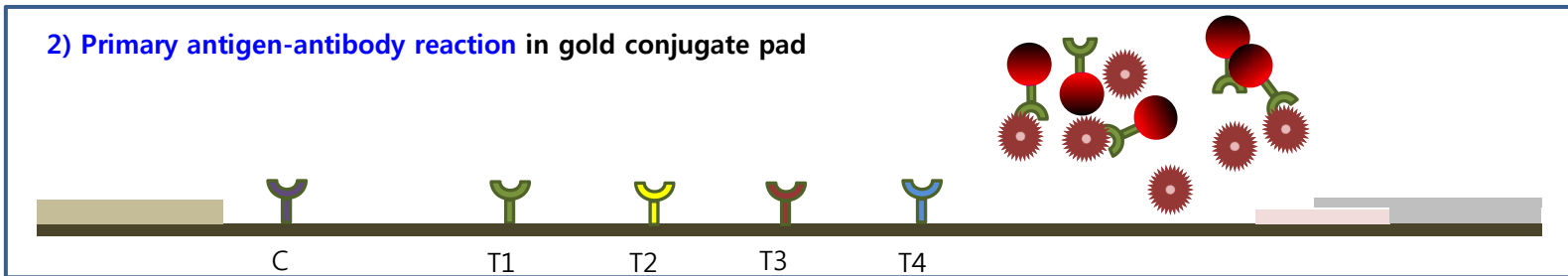


# Principle (Lateral flow immunochromatographic assay)

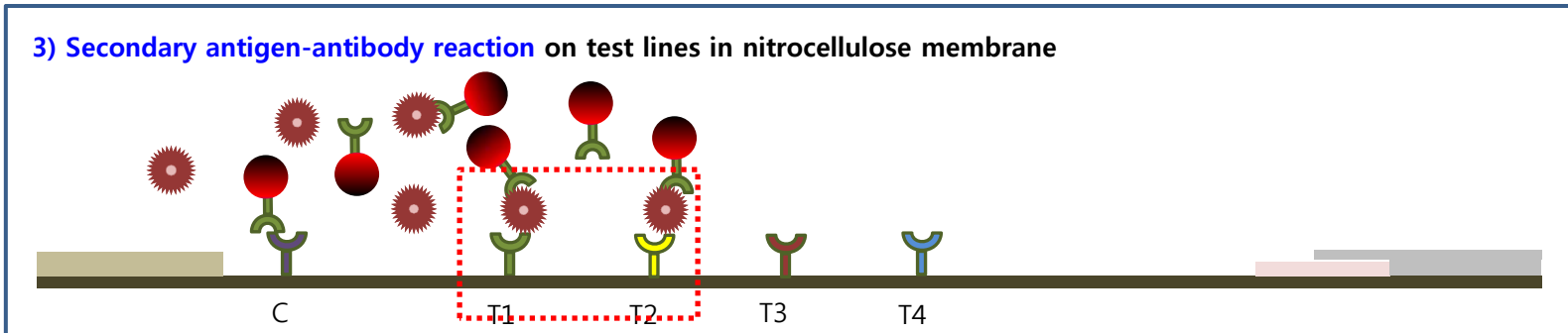
## 1) Sample loading



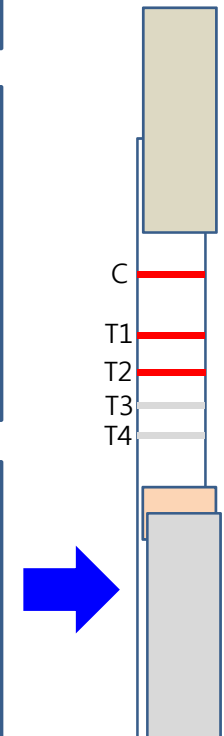
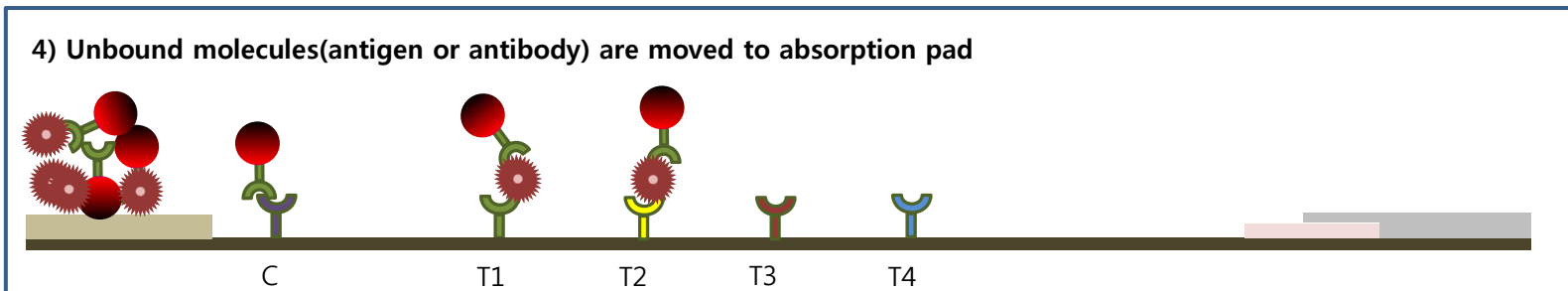
## 2) Primary antigen-antibody reaction in gold conjugate pad



## 3) Secondary antigen-antibody reaction on test lines in nitrocellulose membrane

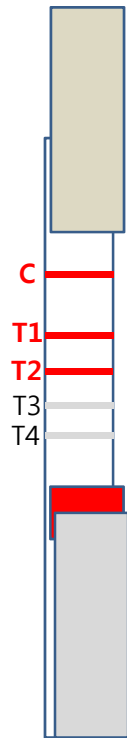


## 4) Unbound molecules (antigen or antibody) are moved to absorption pad

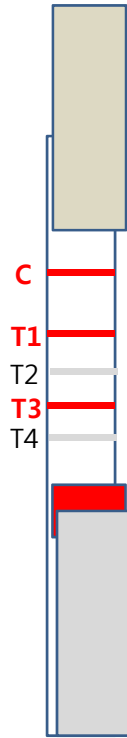


# Strategy

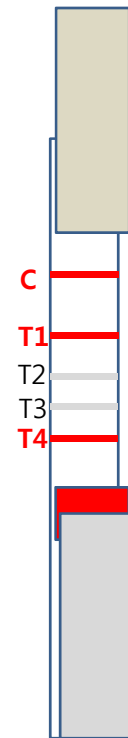
FMDV O (+)



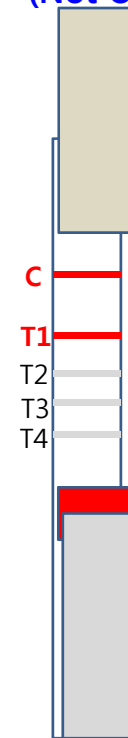
FMDV A (+)



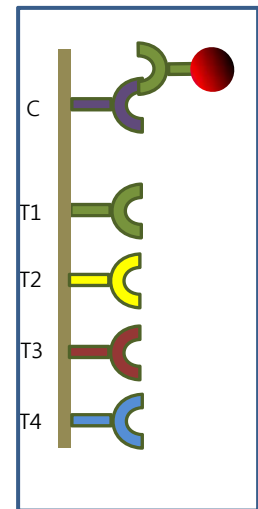
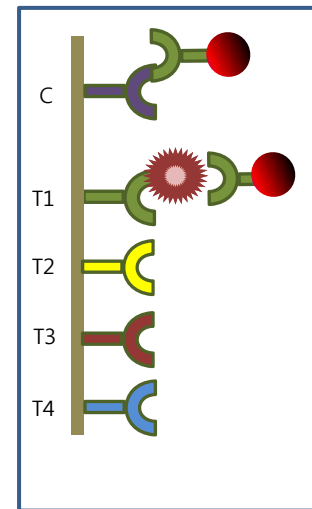
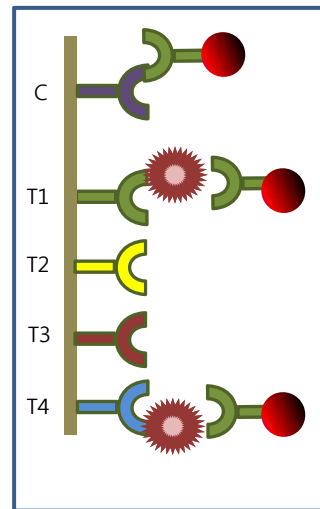
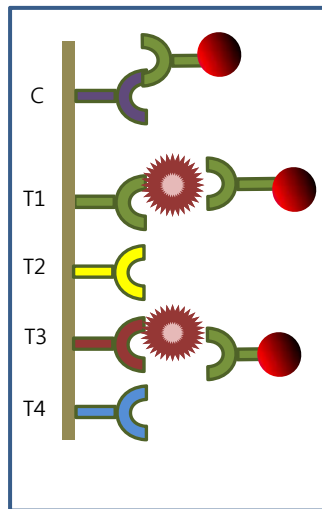
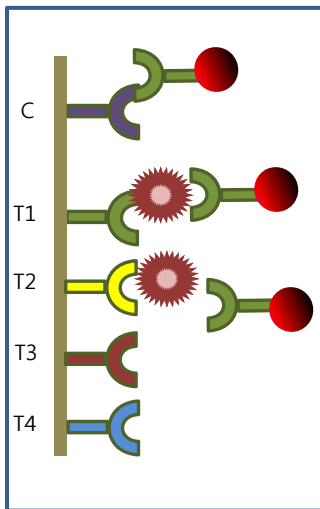
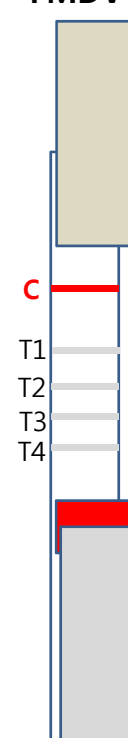
FMDV Asia1 (+)



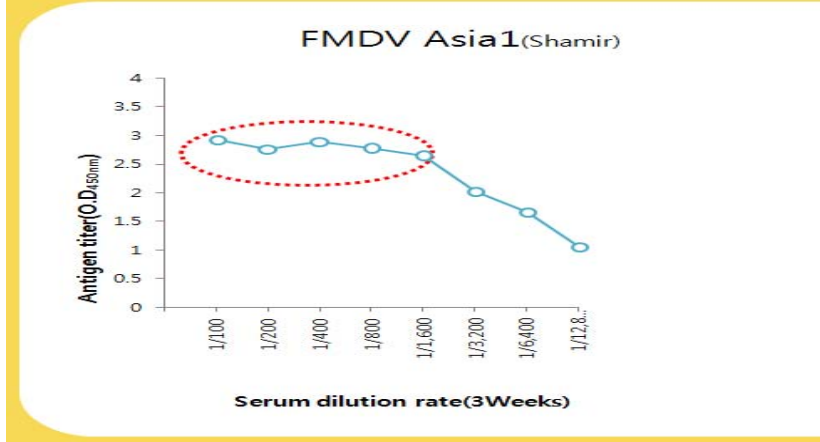
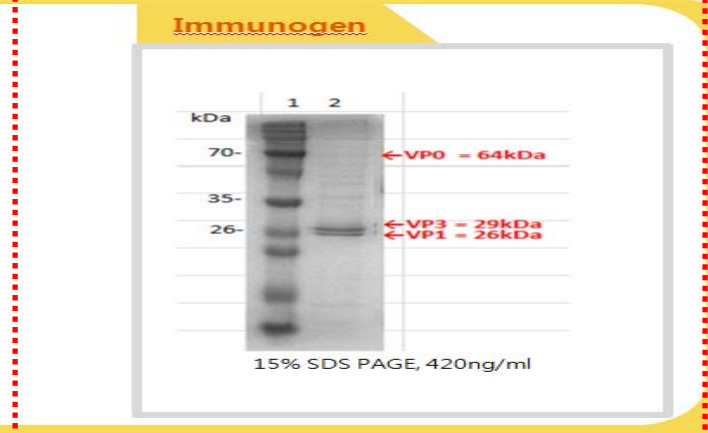
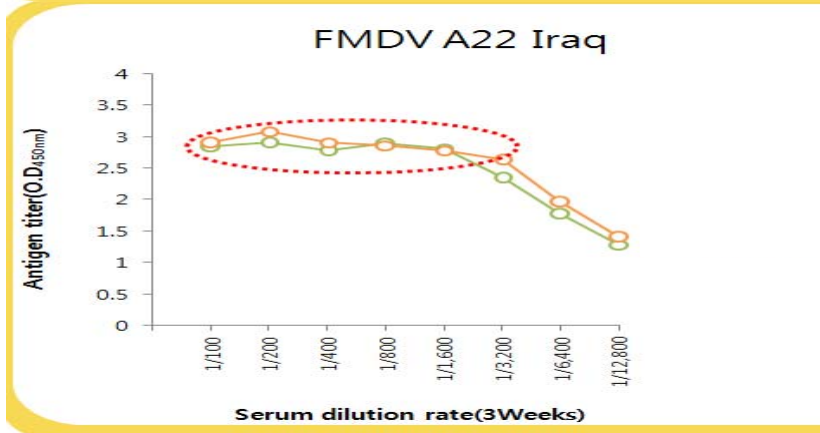
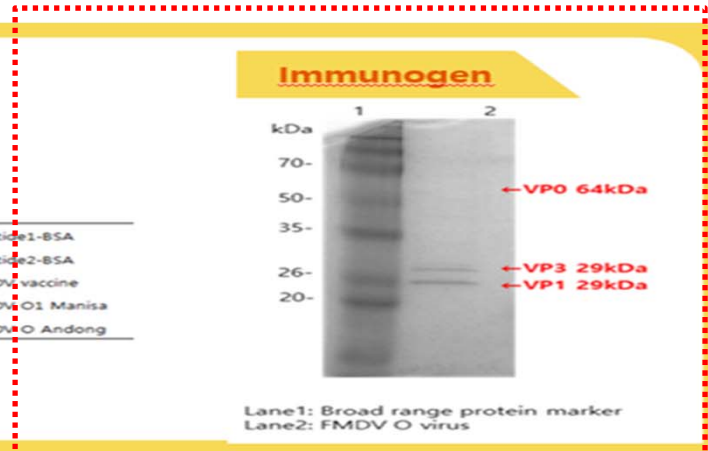
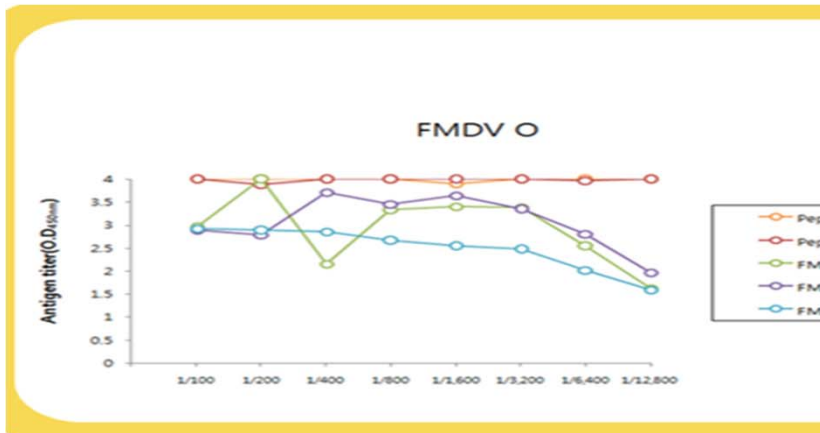
FMDV(+)(C, SAT1-3)  
(Not O, A, Asia1)



FMDV (-)



# Immunogen



# Characterization

## (Capture & Conjugate mAbs)

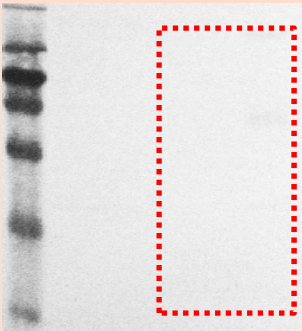
---

Serotype	Immunogen	Clone	Isotype	Epitope	VNT
FMDV O (Capture mAb)	O1 Manisa	7F32	IgG1	Conformational	-
FMDV A (Capture mAb)	A22 Iraq	3H28	IgG1	Conformational	-
FMDV Asia1 (Capture mAb)	Asia 1 Shamir	5D39	IgG2a	Conformational	-
FMDV Common (Capture/Conjugate mAb)	A22 Iraq	1E58	IgG1	Conformational	-

---

FMDV Common mAb  
(1E58)

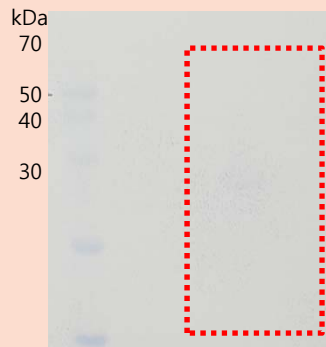
1' Ab 1E58



M E.coli Baculo VP1 VP3 VP0  
P1

FMDV O mAb  
(7F32)

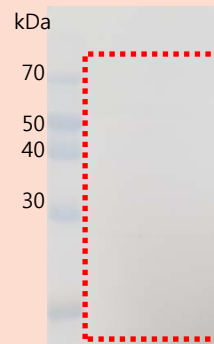
1' Ab 7F32



M E.coli Baculo VP1 VP3 VP0  
O P1

FMDV A mAb  
(3H28)

1' Ab 3H28



M E.coli Baculo  
A P1

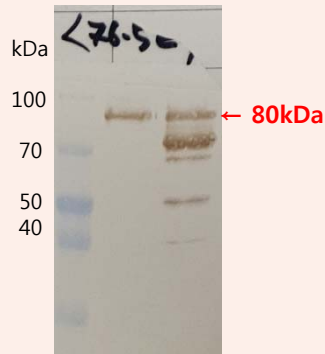
FMDV Asia1 mAb  
(5D39)

1' Ab 5D39



M E.coli Baculo  
Asia P1

PC: 1' Ab O type 76.5E



M E.coli Baculo  
O P1



# Characterization

## (Capture & Conjugate mAbs)

Serotype	Immunogen	Clone	Isotype	Epitope	VNT
FMDV O (Capture mAb)	O1 Manisa	7F32	IgG1	Conformational	-
FMDV A (Capture mAb)	A22 Iraq	3H28	IgG1	Conformational	-
FMDV Asia1 (Capture mAb)	Asia 1 Shamir	5D39	IgG2a	Conformational	-
FMDV Common (Capture/Conjugate mAb)	A22 Iraq	1E58	IgG1	Conformational	-

# Validation

## (Reactivity/Specificity)

Serotype	O									A		Asia1			C	SAT			Neagive Control		
Topotype	SEA /Mya-98	SEA /Mya-98	ME-SA /PanAsia	ME-SA /PanAsia	ME-SA /ind-2001d	ME-SA	Euro-SA	Cathay	Cathay	Asia /Sea-97	Asia/G-IV	G-V			Euro-SA	SAT1	SAT2	SAT3	seneca valley virus	Swine vesicular disease	cell culture supe.
Name	전천	베트남 (VN1)	Chung-Ju	O1 /SKR/2002	보은1차	O1manisa	O1/BFS/86/UK/87	Yunlin Taiwan/97	O8089	포천주	A22 Iraq	MOG/05	CAM/9/80	Sharmir	C3/Resend e/BRA/65	SAT1 /BOT/1/68	SAT2 /ZIM/5/61	SAT3 /ZIM/4/61	SVV	PiC-77	NC
Control																					
Common																					
O																					
A																					
Asia1																					

# Validation (Sensitivity)

Sample	A (pochen)			A (yencheon)			O (Boen)			O (Jeongeup)			Asia1 (Sharmir)			C C3 (/Resende/)			SAT1			SAT2			SAT3		
	Dilution (10TCID50)	5	4	3	5	4	3	5	4	3	5	4	3	5	4	3	5	4	3	5	4	3	5	4	3	5	4
Control																											
Common																											
O																											
A																											
Asia1																											
(5 LINE)																											
Detect																											
	+	+	-	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
rRT-PCR	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Sensitivities ranged from  $10^3$  to  $10^4$  TCID<sub>50</sub> of Seven serotypes of FMDV.

# Comparison
















(Svanodip, PBM, Ag-ELISA(Pirbright), rRT-PCR)

Sample	A (pocheon)			A (Yeoycheon)			O (Boeun)			O (Jeongup)			Asia1 (Sharmir 1)			C (Resende/BRA)			SAT1			SAT2			SAT3		
Dilution (10TCID50)	5	4	3	5	4	3	5	4	3	5	4	3	5	4	3	5	4	3	5	4	3	5	4	3	5	4	3
<b>Detect</b>	+																										
Control Common O A Asia1 (5 LINE)																											
Control Common SVANODIP (2 Line)																											
Control SP NSP (PBM 3 LINE)																											
Ag ELISA (Pirbright)	2.7398	0.5497	0.0663	2.7517	1.1207	0.1356	0.855	0.2349	0.0622	1.0926	0.7026	0.0542	1.8792	0.6027	0.0518	4	2.8357	0.4214	3.5476	2.8212	0.3768	3.2702	1.5022	0.2154	-	-	-
rRT-PCR	+																										

Svanodip: Commercial product, detect all seven serotypes of FMD(Not distinguish)

PBM: Commercial product, detect 4 serotypes of FMD(A, O, Asia1 and C)(Not distinguish)

# Comparison of Visibility

Sample		O Jincheon		
Titer		$10^4$ TCID <sub>50</sub>		
Kind of Kit		APQA 5 LINE	SVANODIP	PBM
MARKS	Control			
	Common			
	O			
	A			
	Asia1			

# Clinical samples (Saliva)

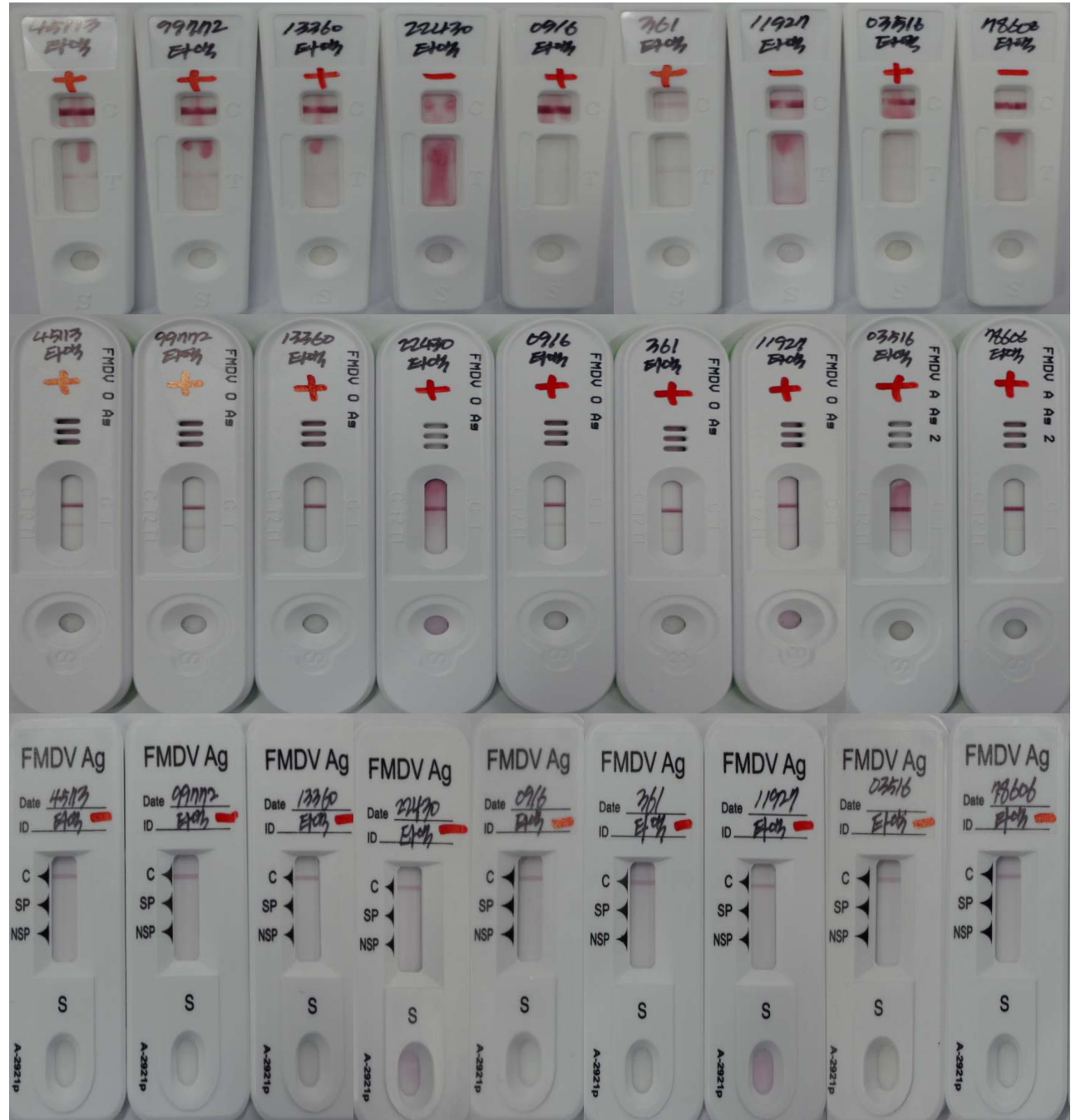
Type		O Type ('17 Outbreaked)		O Type (Infected)	
		Saliva 266	Saliva 439	Saliva A-1	Saliva A-2
<b>Detect</b>		-	+	+	+
<b>Control Common O A Asia1 (5LINE)</b>	Control				
	Common				
	O				
	A				
<b>Control Common SVANODIP(2 LINE)</b>	Control				
	FMDV				
		-	+	W+-	-
<b>Control SP NSP PBM(3LINE)</b>	Control				
	SP				
	NSP				
		-	-	-	-
<b>Ag ELISA(Pirbright)</b>		0.0964	0.1004	0.1026	0.0896
		-	+	+	-
<b>rRT-PCR</b>		+	+	+	+

# Saliva samples(FMDVO)

Pan-FMDV Rapid kit  
SVANODIP(Sweden)  
**(6/9)**

FMDV O Rapid kit  
Korea(APQA)  
**(9/9)**

Pan-FMDV Rapid kit  
PBM(USA)  
**(0/9)**



## Saliva samples(FMDV A)

Pan-FMDV Rapid kit  
PBM(USA)  
**(0/2)**



FMDV A Rapid kit  
Korea(APQA)  
**(2/2)**



Pan-FMDV Rapid kit  
SVANODIP(Sweden)  
**(1/2)**

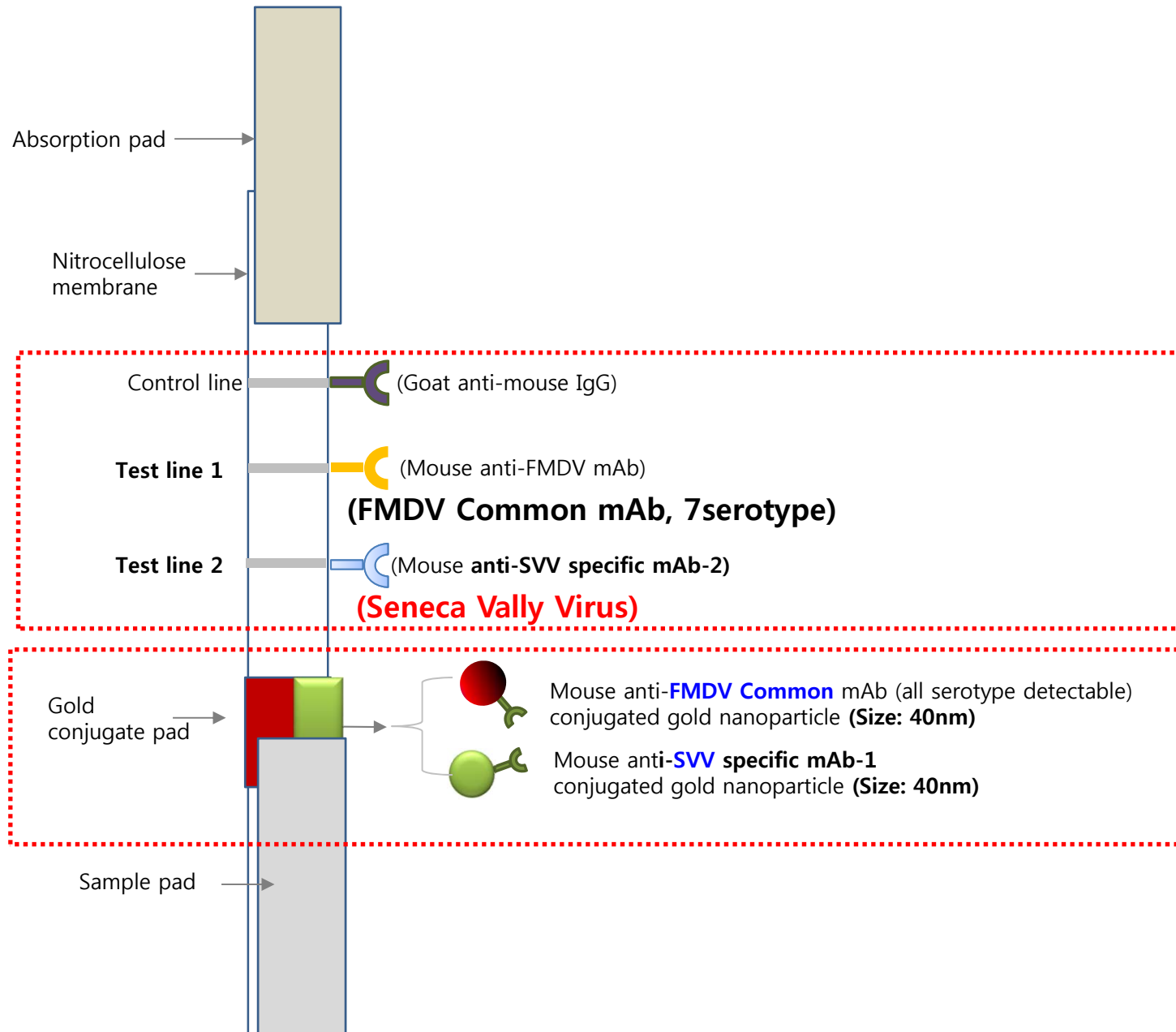




# Conclusions

- FMDV Serotyping strip **can**
  - Detect** all seven serotypes of FMD
  - Distinguish** serotypes O, A and Asia 1
- FMDV Serotyping strip **showed high**
  - Sensitivity**(  $10^{3\sim 4}$  TCID<sub>50</sub>)
  - Accuracy** (rRT-PCR, Ag ELISA)
- FMDV Serotyping strip **will be useful**
  - Where Lab **diagnosis** is **not available**
  - for the **on-site** diagnosis of suspected FMD outbreaks
  - in **Seneca Valley Virus** infected countries

# Further research



# Acknowledgement





THANK  
YOU