



Canadian Food
Inspection Agency

Agence canadienne
d'inspection des aliments

Nuevas Herramientas Serológicas para la Detección de Anticuerpos No-estructurales de Fiebre Aftosa.

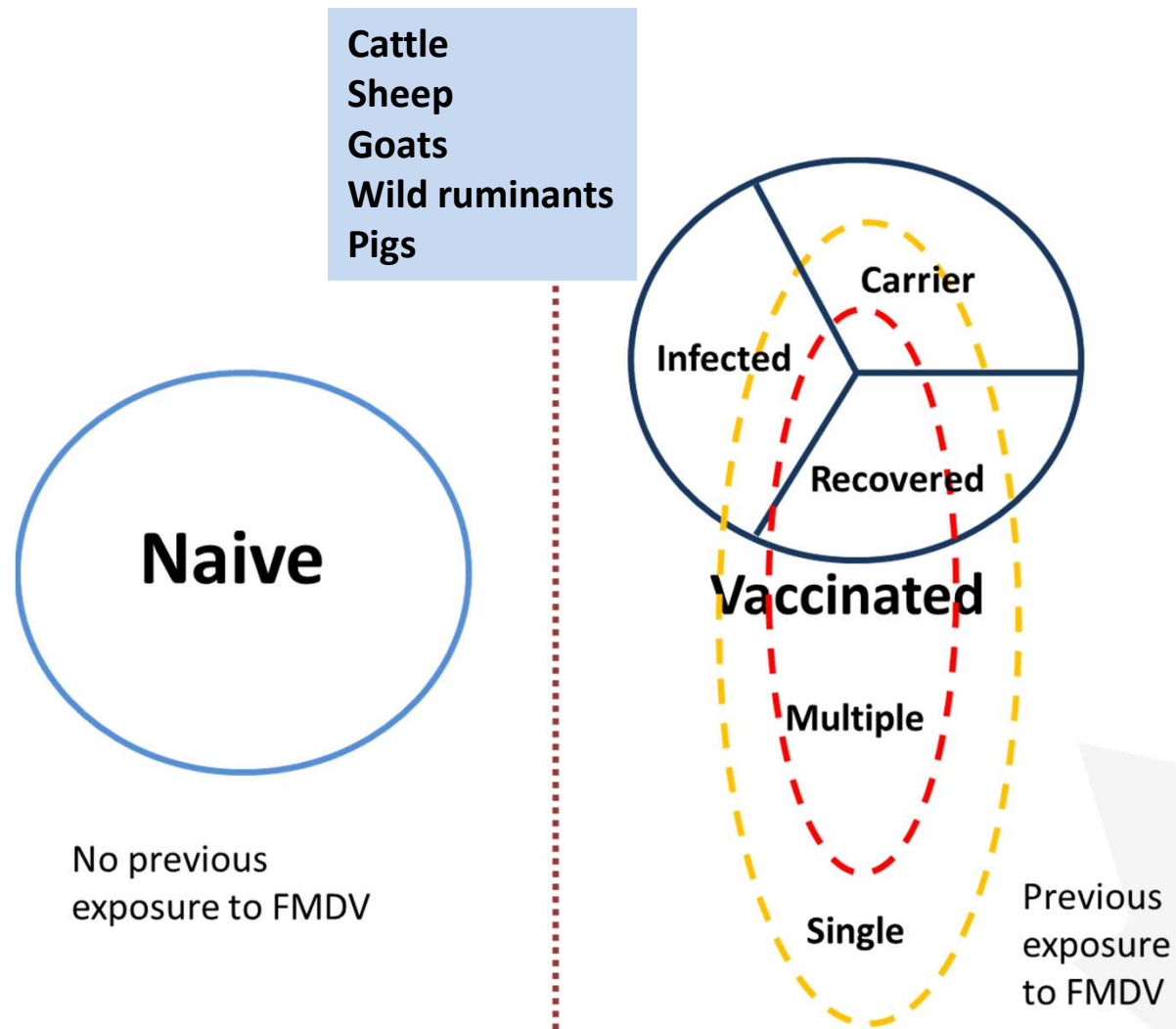
Dr. Alfonso Clavijo
Laboratory Executive Director
National Centers for Animal Diseases
Winnipeg, MB, Canada.



Seminario Pre-COSALFA
Abril 30, 2019

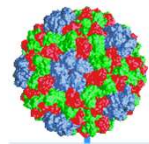
Canada 

Serological States in FMD

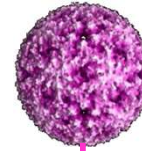


3ABC ELISA

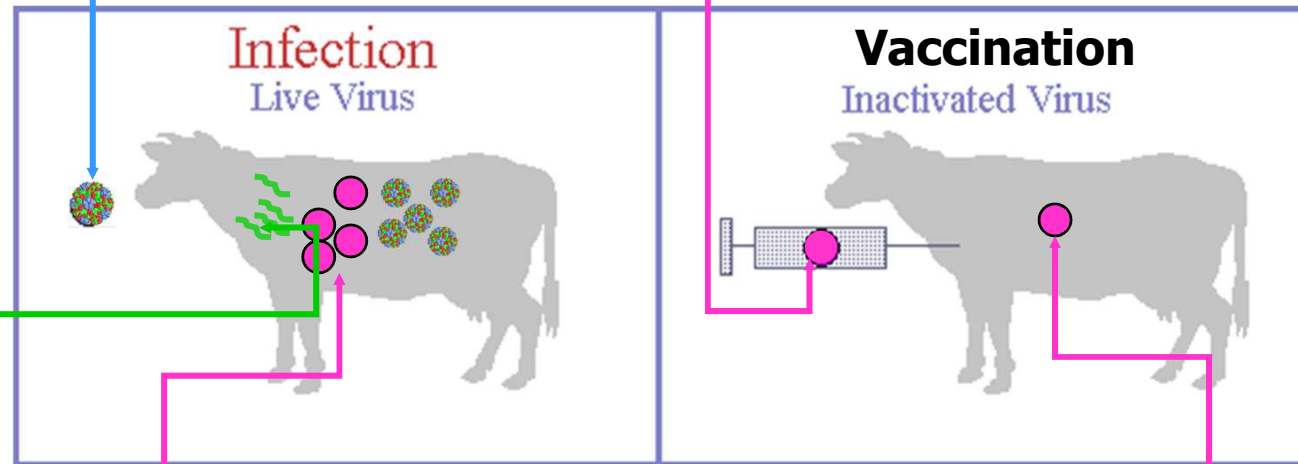
Serotype independent



Live virus



Inactivated Virus

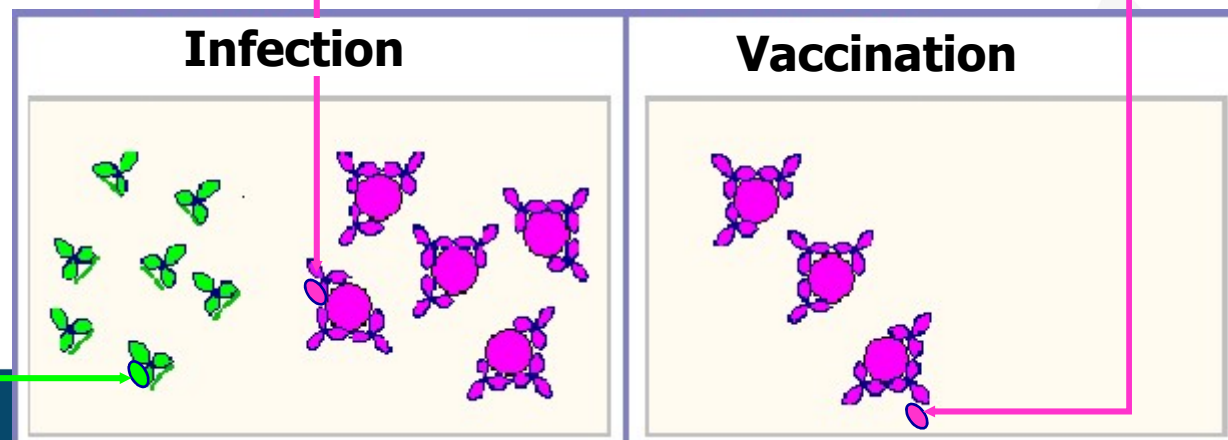


Non-Structural proteins (NSPs)

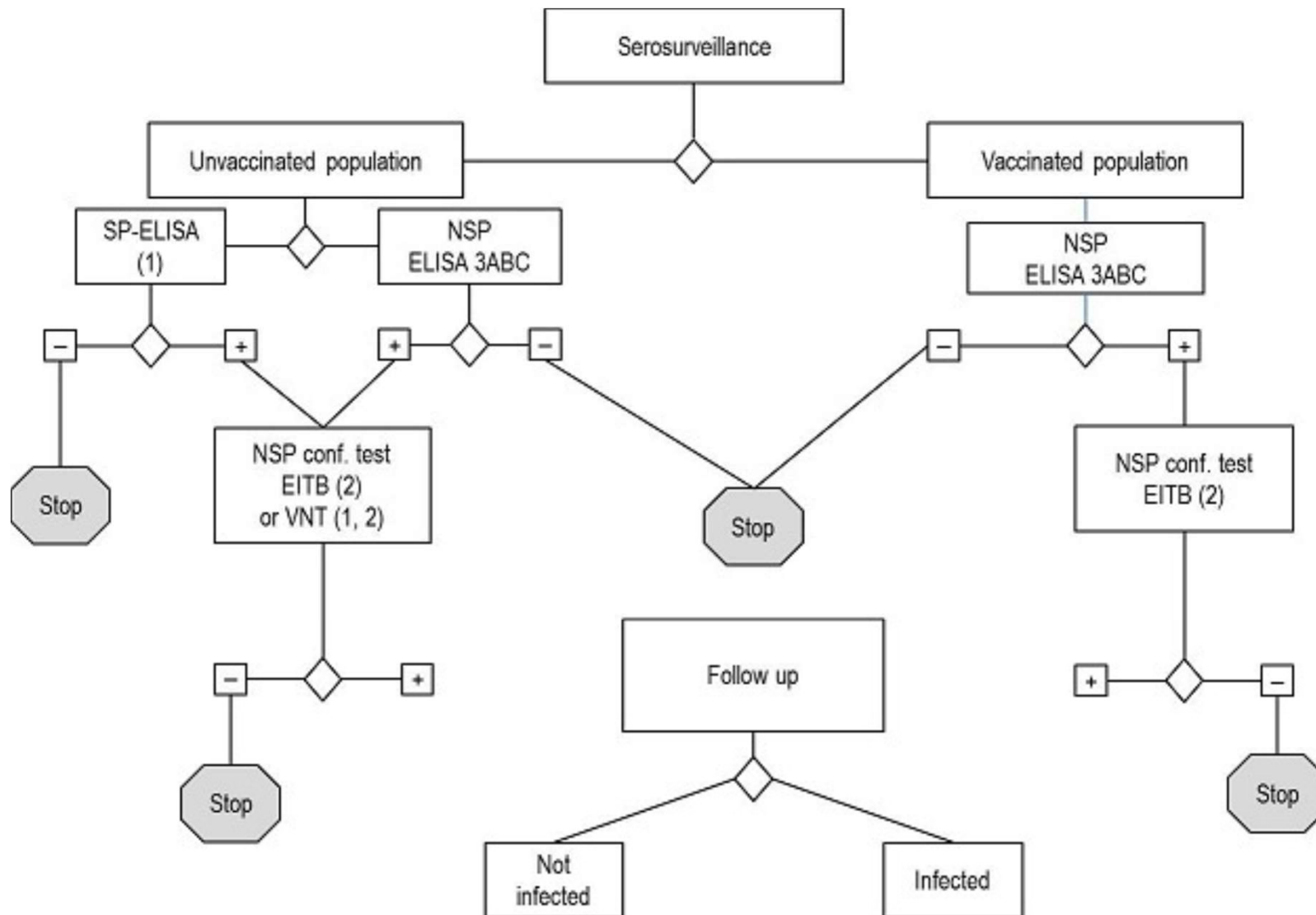
Structural Proteins (SPs)

Anti-SPs

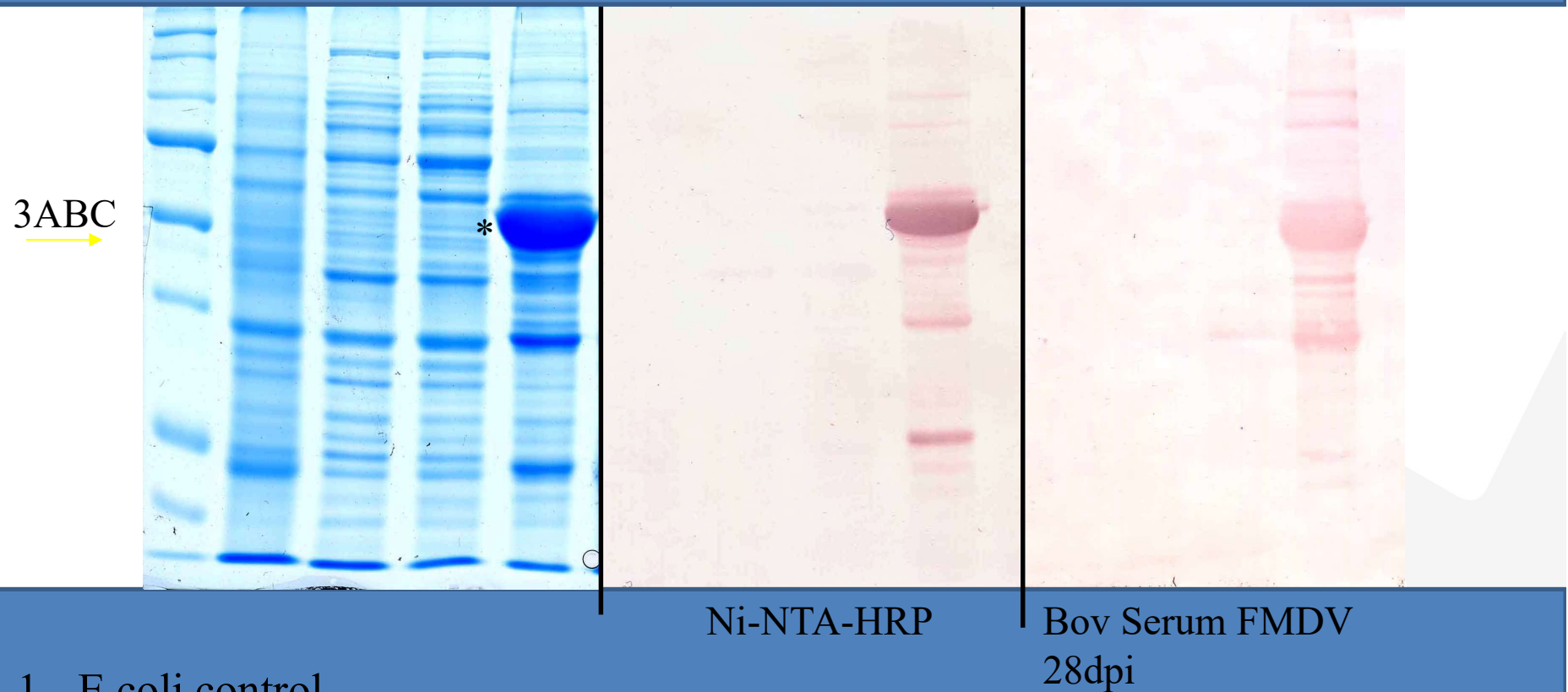
Anti-NSPs



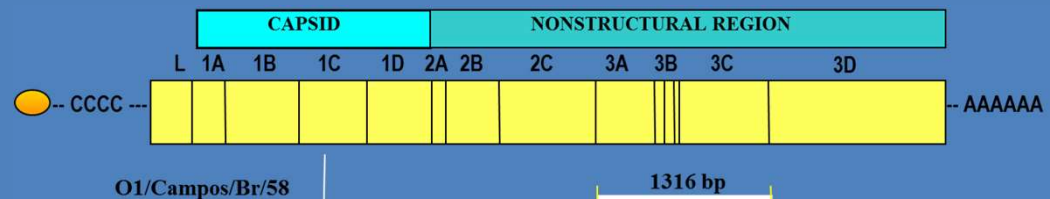
laboratory tests for determining evidence of FMDV infection through or following serological surveys



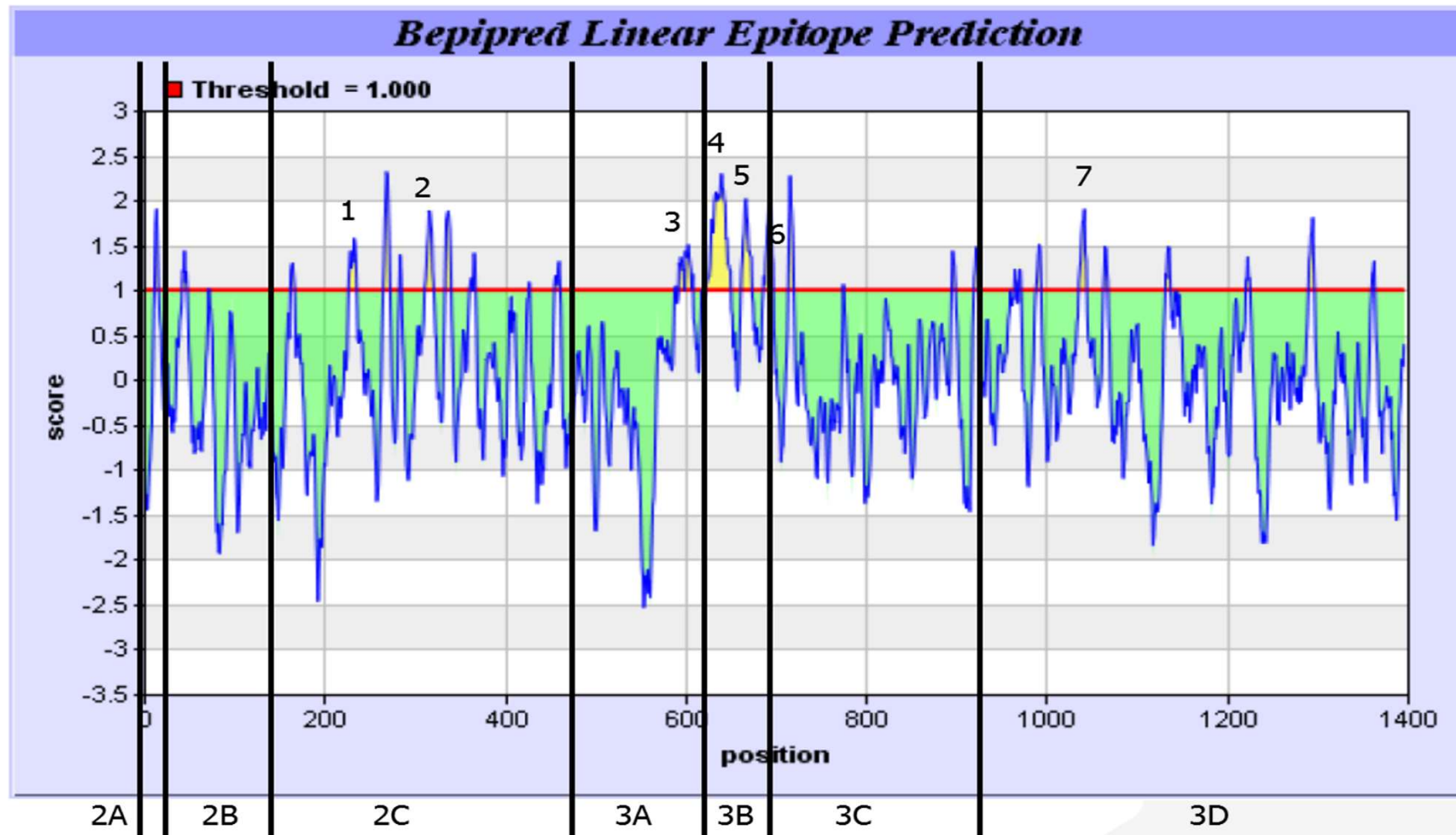
Recombinant protein 3ABC



1. E.coli control
2. BL21(DE)PlysS No induced
3. BL21(DE)PlysS induced:
Soluble fraction
4. Insoluble fraction



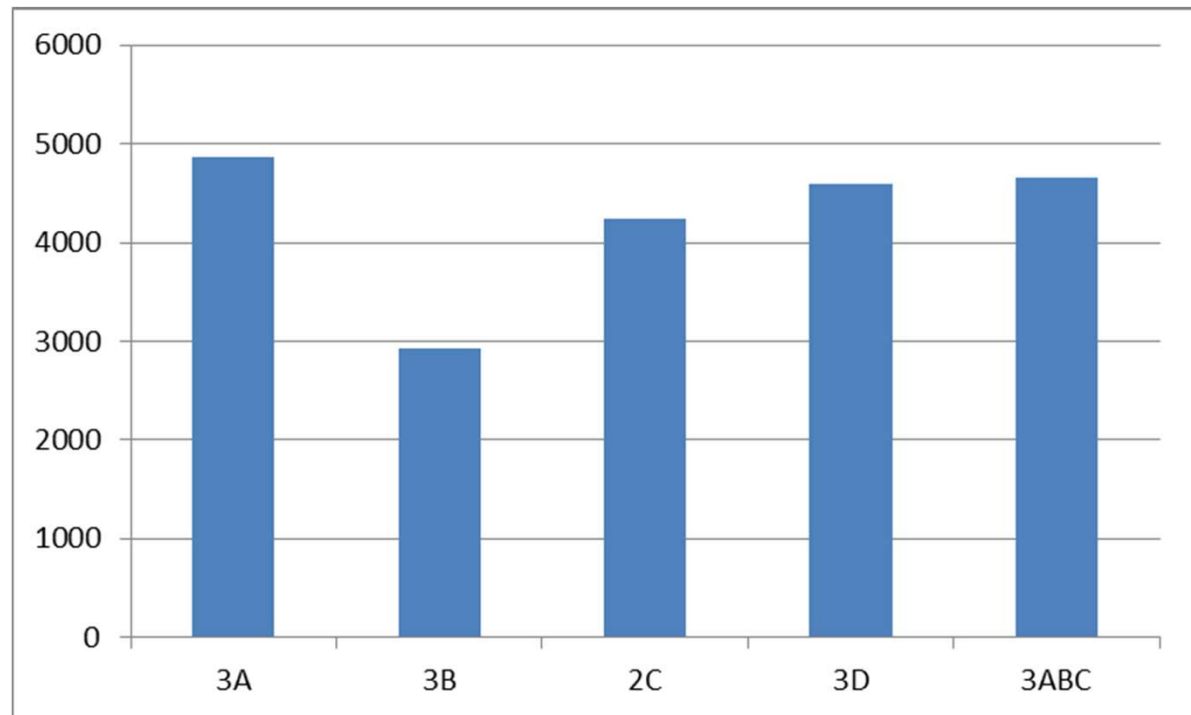
NSP B-cell epitope prediction (linear epitopes)

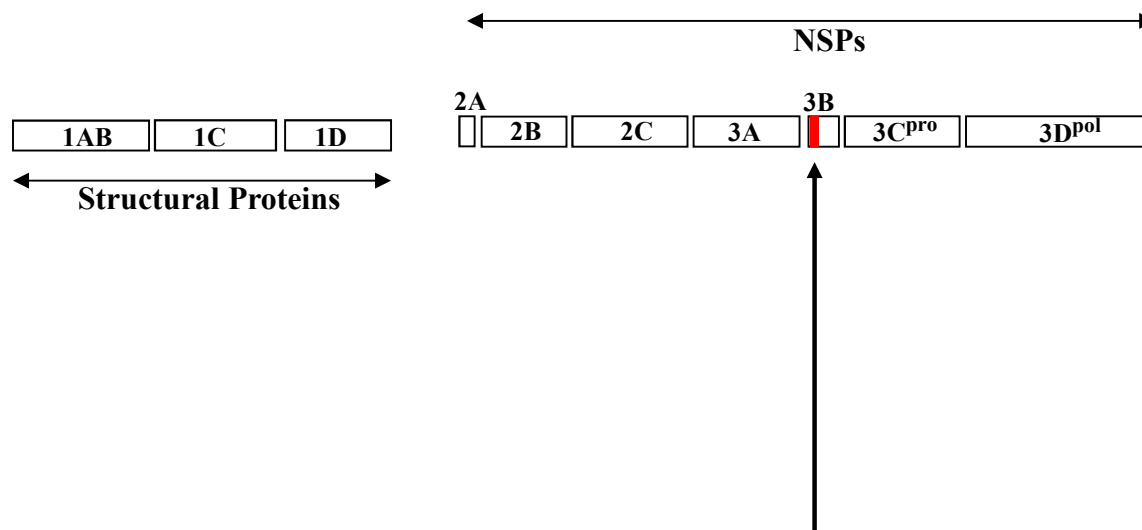
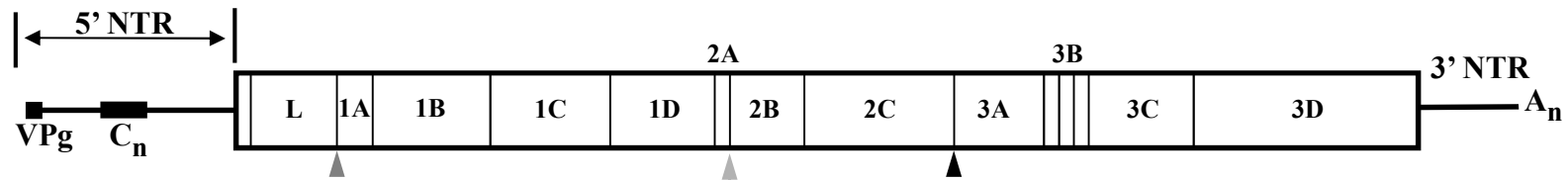


No.	Start	End	Peptide	Length
1	226	234	DLNDPSKYK	9
2	310	320	YCPDPDHFDG	11
3	592	607	KTLDEAEKSPLETSGA	16
4	622	649	ACDDVNSEPAQPVVEEQPQAEGPYAGPLE	28
5	661	672	PQQEGPYAGPME	12
6	686	696	VKEGPYEGPVK	11
7	1035	1043	AMEPDTAPG	9

Reactivity to NSPs in EITB (6,184 cattle. PANAFTOSA) Vaccinated population.

Reactivity	
3A	4871
3B	2929
2C	4246
3D	4597
3ABC	4661
Total	6184

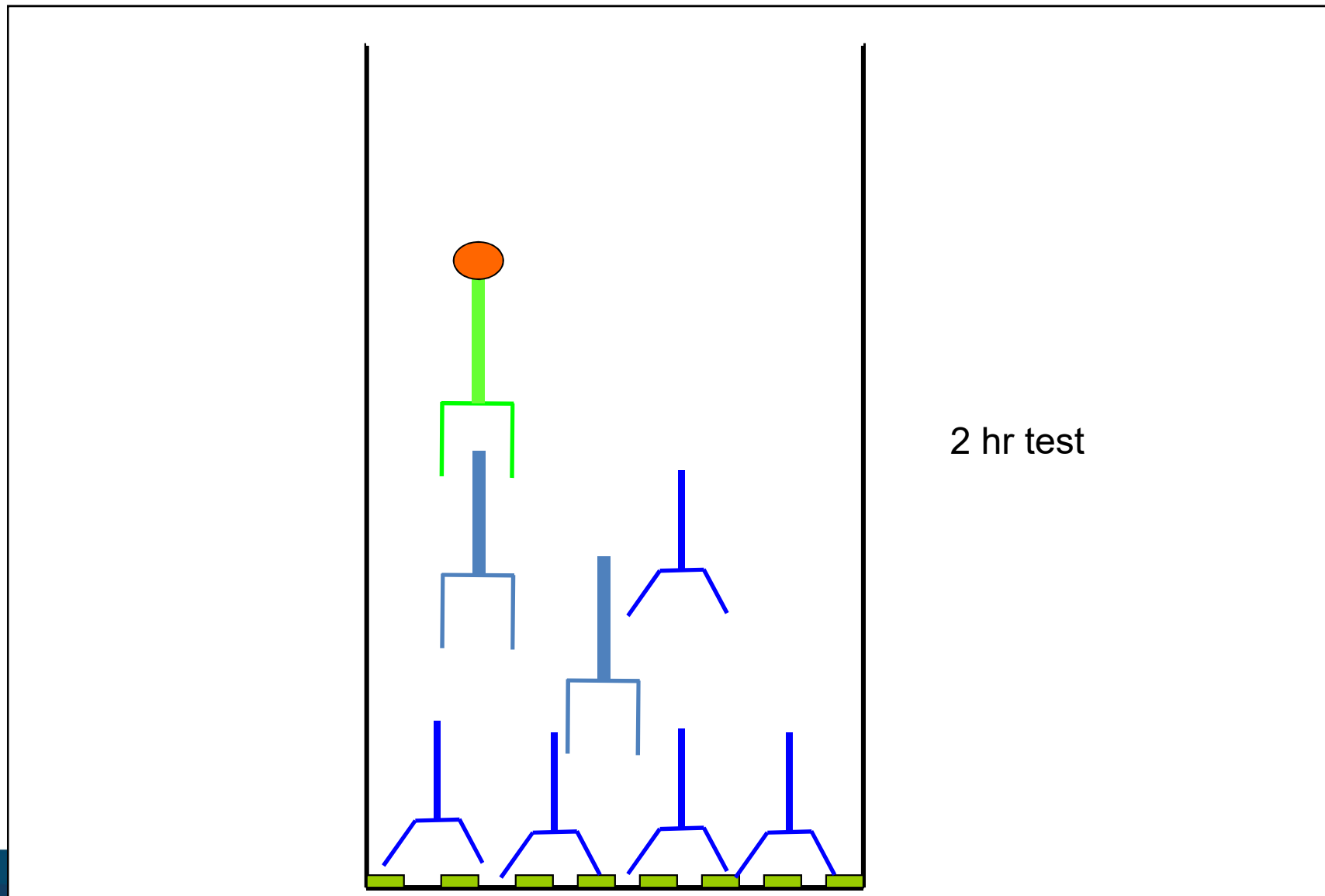




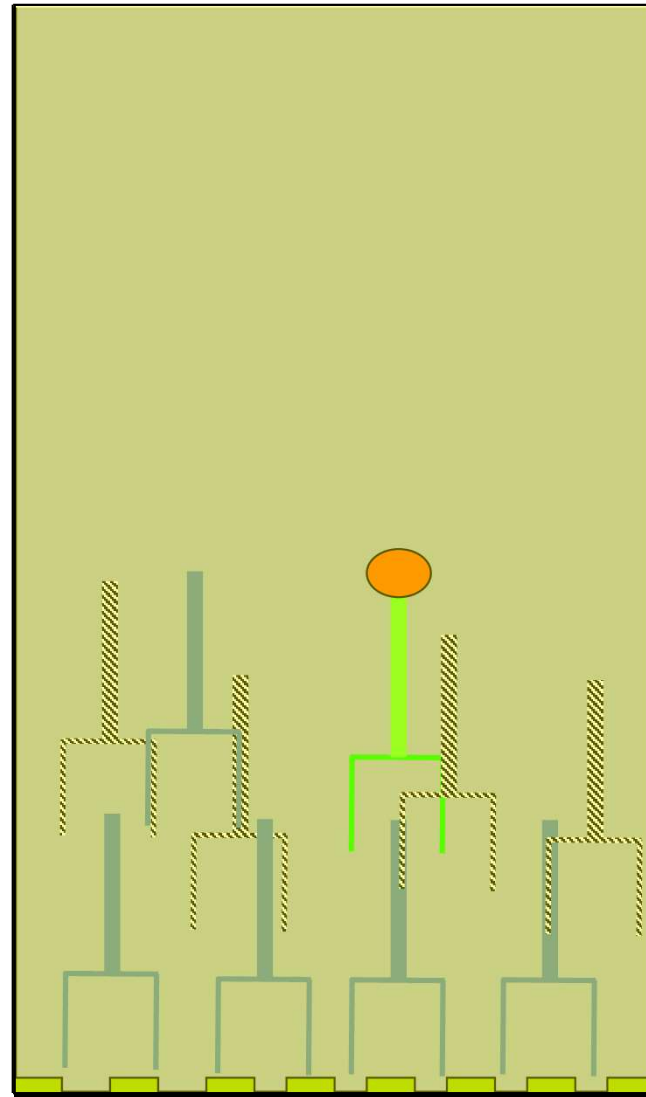
NH₂-CGPYAGPLERQKPLK-COOH

Hohlich BJ, Wiesmuller KH, Schlapp T, Haas B, Pfaff E, Saalmuller A 2003. Identification of foot-and-mouth disease virus-specific linear B-cell epitopes to differentiate between infected and vaccinated cattle. J Virol.;77(16):8633-9

3ABC Solid Phase Blocking ELISA (Positive)



3ABC Solid Phase Blocking ELISA (Negative)



2 hr test



Organização
Pan-Americana
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SECRETARIA REGIONAL PARA AS Américas

PANAFTOSA
Centro Pan-Americano de Febre Aftosa
Saúde Pública Veterinária



PANAFTOSA / SPV
OPS / OMS



PILOTO bELISA (3ABC) Caja 1

Piloto bElisa (3ABC) - Caja 1
Prueba Confirmatoria (EITB) - Caja 2
1 Kit = 440 Pruebas Tamiz

Kit para detección de anticuerpos anti-3ABC (VFA)
a través de la técnica inmunoenzimática -ELISA en microplaca.

COMPONENTES

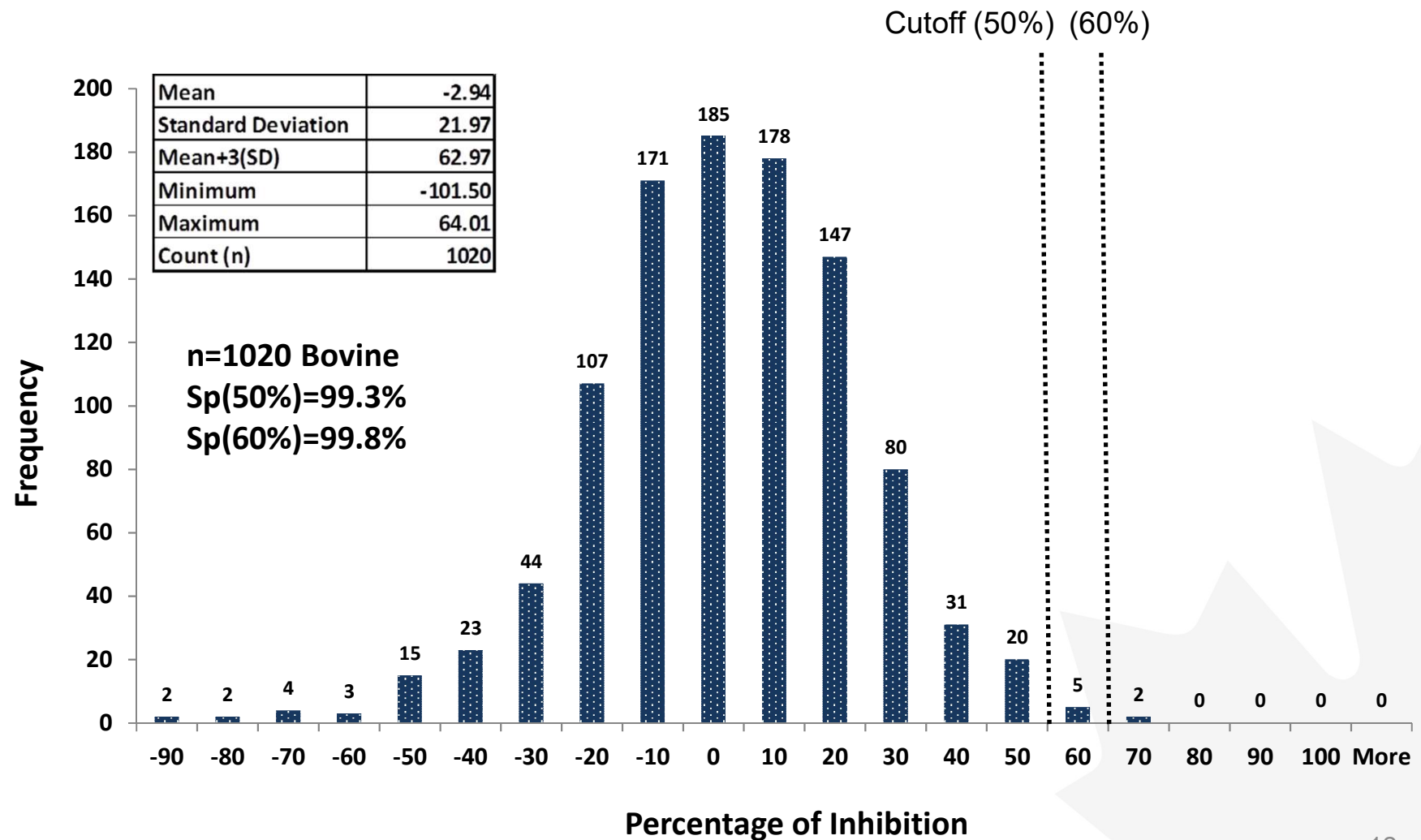
CANTIDAD

Conjugado	1 (960 µL)
Control Negativo	1 (600 µL)
Control Positivo 1	1 (600 µL)
Control Positivo 2	1 (600 µL)
Antecuerpo Monoclonal	1 (720 µL)
Microplacas	10 (96 pocillos c/u)
Buffer de Lavado 25 x	2 (130 mL)
Sello de Placas (adhesivos)	10 (unidades)
Solución Bloqueadora	1 (60 mL)
Sustrato	1 (60 mL)

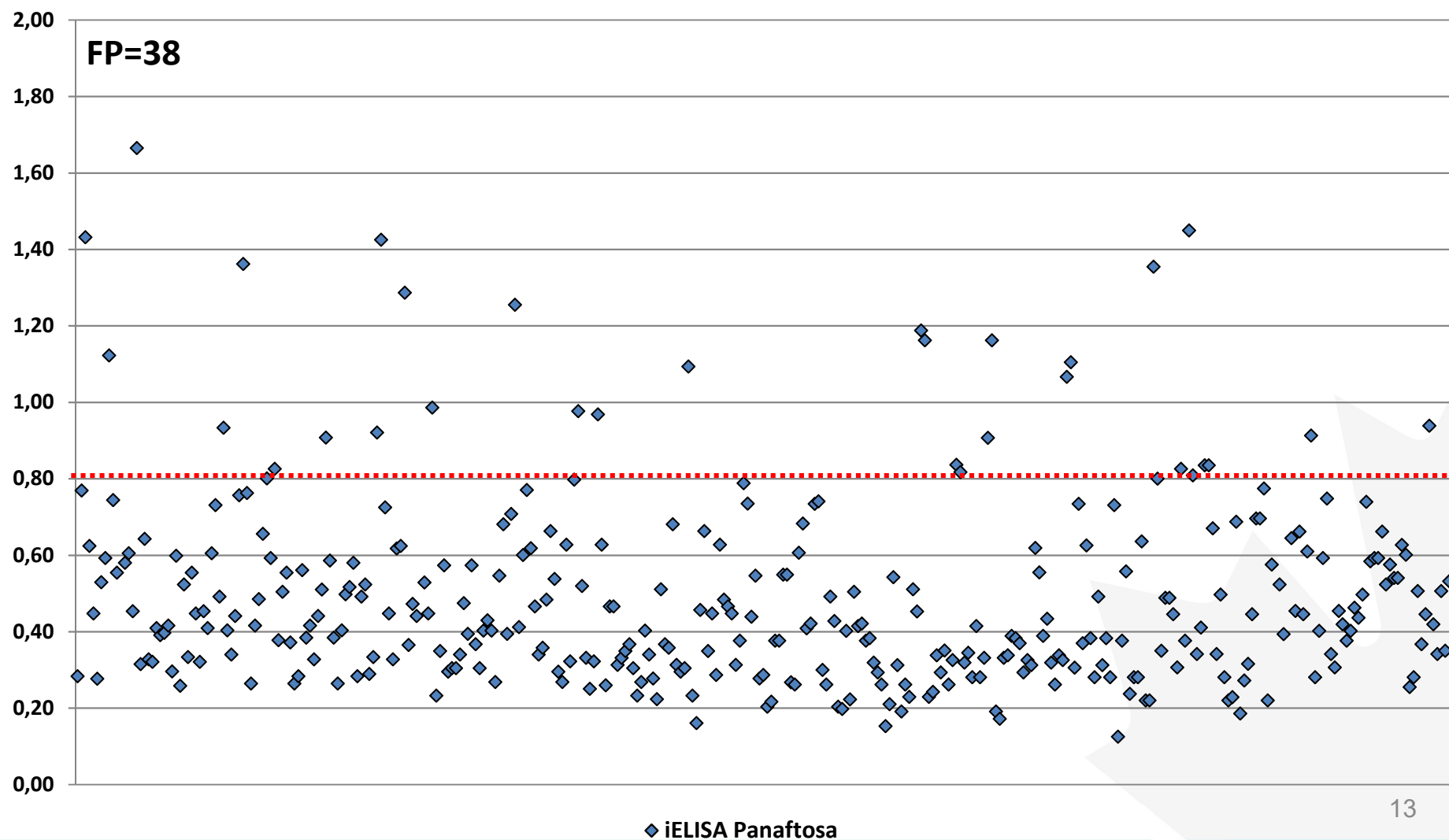
Uso sólo para diagnóstico. Mantener refrigerado de 2 a 8 ° C.
Antes de comenzar la prueba, lea las instrucciones adjuntas.

Fab: 03/19
Venc: 03/20
Lote: FAP0119111

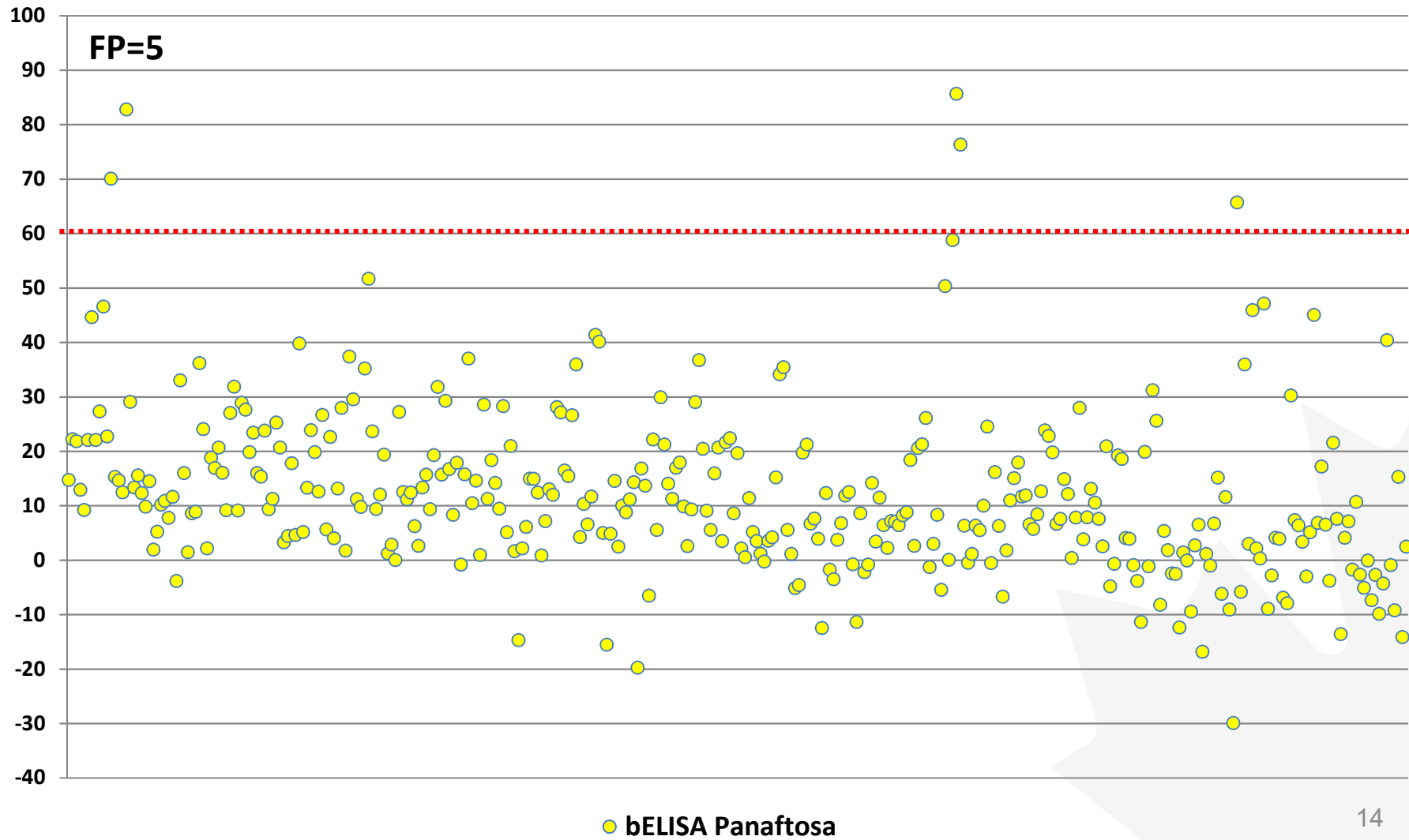
3ABC bELISA distribution of Cattle Negative Samples



3ABC bELISA distribution of vaccinated Cattle (13-24; 25-36; >36) iELISA Panaftosa (n=350)



3ABC bELISA distribution of vaccinated Cattle (13-24; 25-36; >36) bELISA Panaftosa (n=350)



3ABC iELISA Vs bELISA Panaftosa Vaccinated Cattle (13-24; 25-36; >36)

	iELISA			bELISA 60%			
Edad meses	Pos	Neg	DxSp	Pos	Neg	DxSp	Total
13-24	10	92	90.19	3	99	97.05	102
25-36	7	71	91.02	1	77	98.71	78
>36	21	149	87.64	1	169	99.41	170
Total	38	312	89.14	5	345	98.39	350

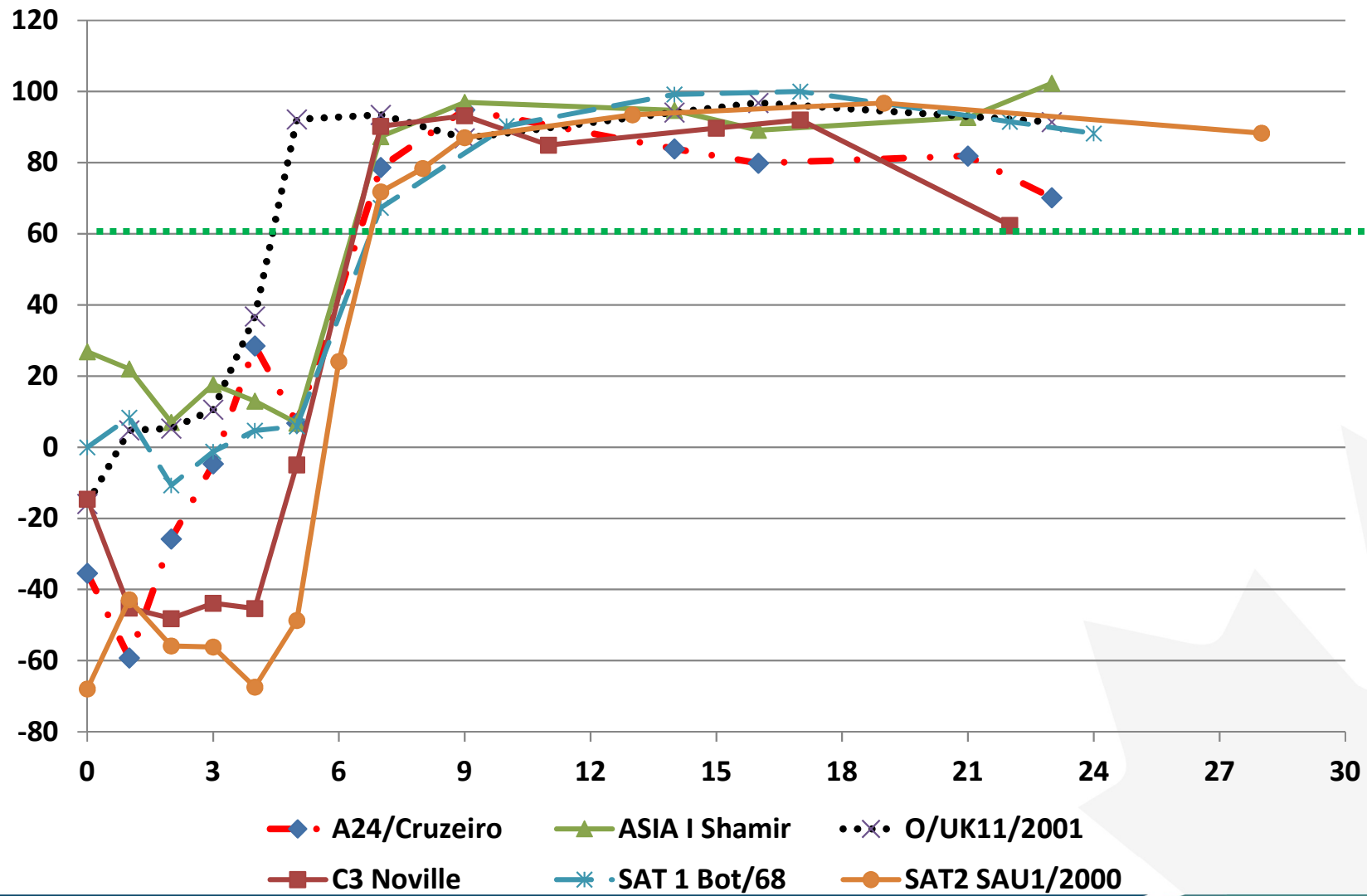
3ABC bELISA experimentally infected Cattle

	PrioCheck	bELISA
A24 Cruzeiro: C140-DPI 0	2.9	-35
A24 Cruzeiro: C140-DPI 1	4.2	-59
A24 Cruzeiro: C140-DPI 2	3.3	-26
A24 Cruzeiro: C140-DPI 3	9.3	-5
A24 Cruzeiro: C140-DPI 4	-3.7	28
A24 Cruzeiro: C140-DPI 5	12.0	7
A24 Cruzeiro: C140-DPI 7	44.7	79
A24 Cruzeiro: C140-DPI 9	67.5	95
A24 Cruzeiro: C140-DPI 14	76.2	84
A24 Cruzeiro: C140-DPI 16	77.2	80
A24 Cruzeiro: C140-DPI 21	78.5	82
A24 Cruzeiro: C140-DPI 23	82.1	70
A24 Cruzeiro: C141-DPI 0	-2.5	-34
A24 Cruzeiro: C141-DPI 1	-0.1	-35
A24 Cruzeiro: C141-DPI 2	1.9	-67
A24 Cruzeiro: C141-DPI 3	-3.4	-49
A24 Cruzeiro: C141-DPI 4	0.6	-69
A24 Cruzeiro: C141-DPI 5	5.3	-59
A24 Cruzeiro: C141-DPI 7	57.6	69
A24 Cruzeiro: C141-DPI 9	68.6	85
A24 Cruzeiro: C141-DPI 14	59.7	103
A24 Cruzeiro: C141-DPI 16	58.6	101
A24 Cruzeiro: C141-DPI 21	60.1	96
A24 Cruzeiro: C141-DPI 23	64.0	94

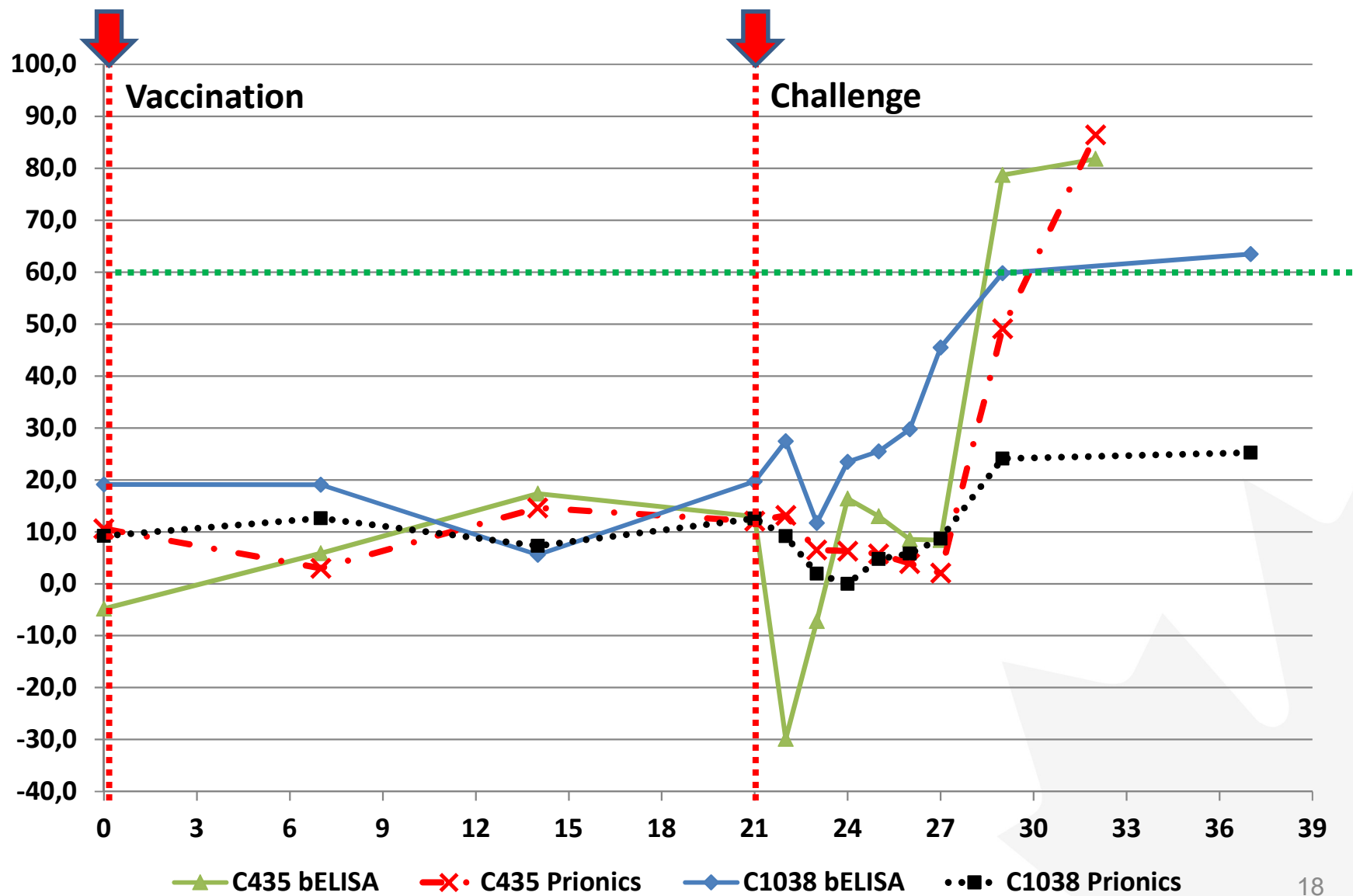
	PrioCheck	bELISA
OUKG11/2001: C138-DPI 0	18.5	12
OUKG11/2001: C138-DPI 1	28.7	19
OUKG11/2001: C138-DPI 2	14.8	71
OUKG11/2001: C138-DPI 3	10.4	31
OUKG11/2001: C138-DPI 4	18.5	39
OUKG11/2001: C138-DPI 5	24.7	71
OUKG11/2001: C138-DPI 7	70.7	86
OUKG11/2001: C138-DPI 9	83.4	93
OUKG11/2001: C138-DPI 14	79.9	82
OUKG11/2001: C138-DPI 16	77.0	86
OUKG11/2001: C138-DPI 23	75.6	87
OUKG11/2001: C138-DPI 28	77.0	87
OUKG11/2001: C139-DPI 1	1.6	-16
OUKG11/2001: C139-DPI 2	-2.8	5
OUKG11/2001: C139-DPI 3	-7.0	5
OUKG11/2001: C139-DPI 4	-9.7	11
OUKG11/2001: C139-DPI 5	13.1	37
OUKG11/2001: C139-DPI 7	46.8	92
OUKG11/2001: C139-DPI 9	52.7	93
OUKG11/2001: C139-DPI 14	49.6	87
OUKG11/2001: C139-DPI 16	60.6	94
OUKG11/2001: C139-DPI 21	58.4	97
OUKG11/2001: C139-DPI 23	62.8	91

	PrioCheck	bELISA
Asia 1 Shamir: C134-DPI 0	3.6	-20
Asia 1 Shamir: C134-DPI 1	3.6	-41
Asia 1 Shamir: C134-DPI 2	4.1	-9
Asia 1 Shamir: C134-DPI 3	0.7	-12
Asia 1 Shamir: C134-DPI 4	-6.4	45
Asia 1 Shamir: C134-DPI 5	7.4	0
Asia 1 Shamir: C134-DPI 7	55.4	72
Asia 1 Shamir: C134-DPI 10	92.0	96
Asia 1 Shamir: C134-DPI 14	93.2	102
Asia 1 Shamir: C134-DPI 17	93.0	101
Asia 1 Shamir: C134-DPI 22	92.8	91
Asia 1 Shamir: C134-DPI 24	92.3	90
Asia 1 Shamir: C145-DPI 0	17.5	27
Asia 1 Shamir: C145-DPI 1	23.8	22
Asia 1 Shamir: C145-DPI 2	21.8	7
Asia 1 Shamir: C145-DPI 3	23.4	18
Asia 1 Shamir: C145-DPI 4	24.2	13
Asia 1 Shamir: C145-DPI 5	28.4	7
Asia 1 Shamir: C145-DPI 7	61.9	87
Asia 1 Shamir: C145-DPI 10	85.5	97
Asia 1 Shamir: C145-DPI 14	78.9	95
Asia 1 Shamir: C145-DPI 17	76.9	89
Asia 1 Shamir: C145-DPI 22	83.3	93
Asia 1 Shamir: C145-DPI 24	85.2	102

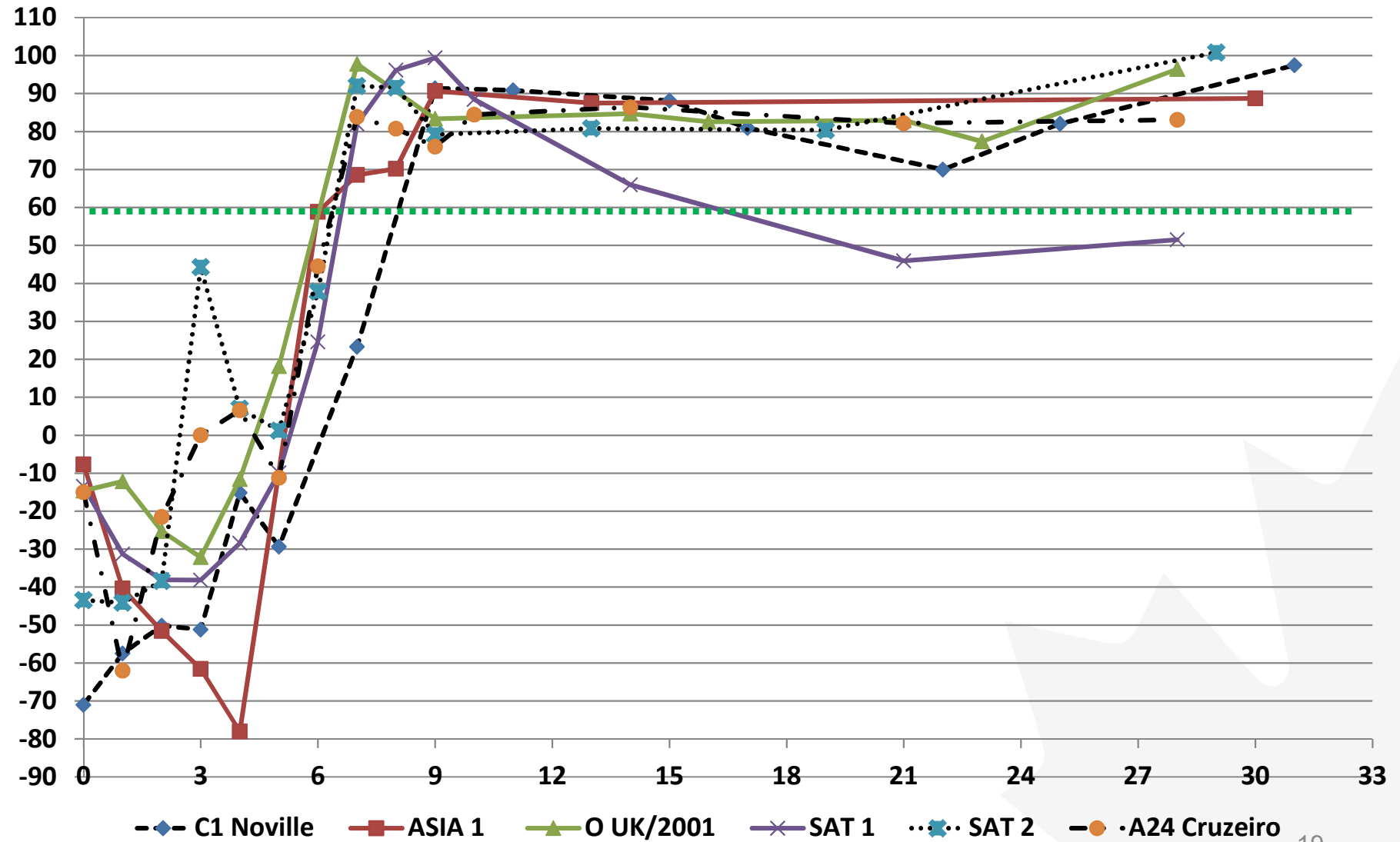
3ABC bELISA experimentally infected Cattle



3ABC bELISA Vaccinated/ Challenged Cattle



3ABC bELISA experimentally infected Sheep



Multiple testing

Parallel testing- Positive in either test **=P**

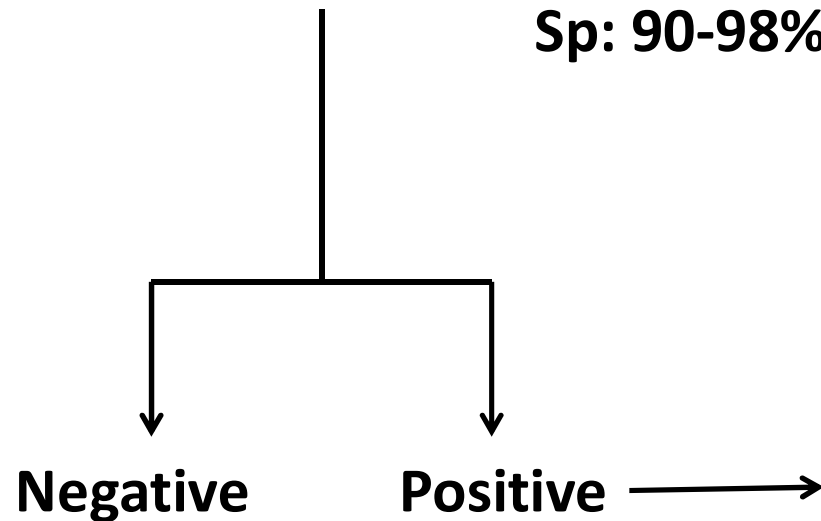
The animal is being asked to “prove” that it is healthy. ↑ Sn and NPV

Serial testing- Positive in all test **=P**

The animal is being asked to “prove” that it has the disease. ↑ Sp and PPV

EITB (Enzyme-linked Immuno-electrotransfer Blot Assay)

ELISA-3ABC: Sn: 98%
Sp: 90-98%

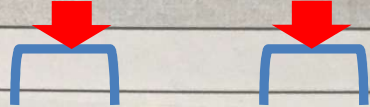
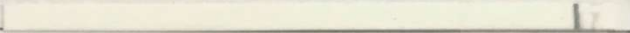
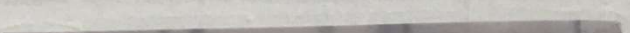
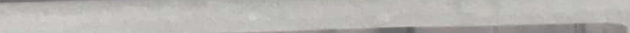


(vaccinated cattle)
Sn: 61% 15-27dpi
23% 28-100 dp

Test with high specificity

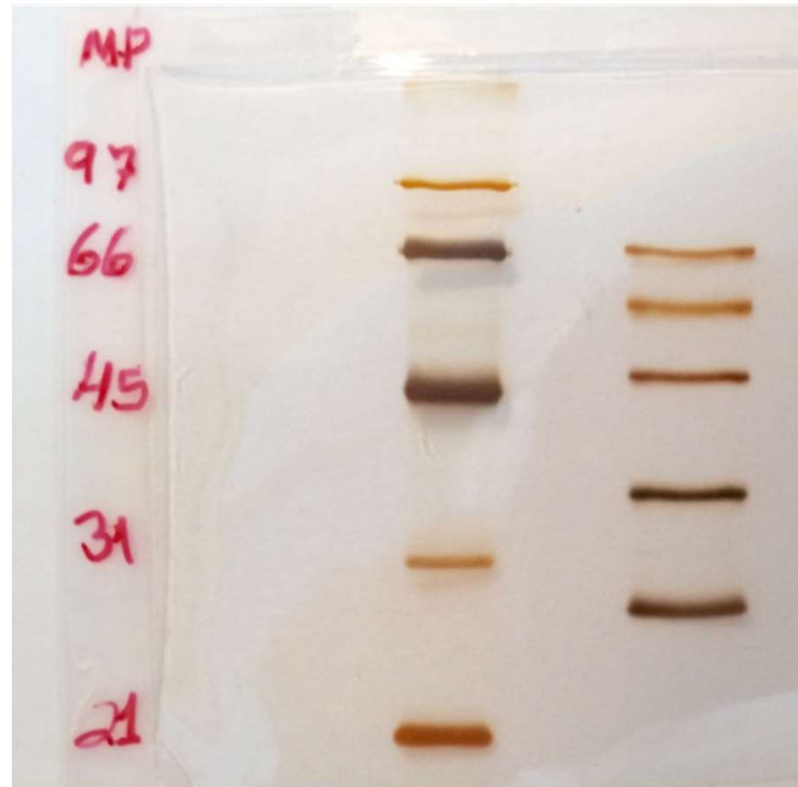
ID	ELISA	3A	3B	2C	3D	3ABC	IDGA
Pos 03							
Pos 02							
Pos 01							
NEG							
1	R						N
2	R						P
3	R						P
4	R						P
5	R						P
6	R						P
7	R						P
8	R						P
9	R						P
10	R						P
11	R						P
12	R						P
13	R						P
14	R						P
15	R						P
16	R						P
17	R						P
18	R						P
19	NR						N
20	R						P
21	R						P

Current EITB issues

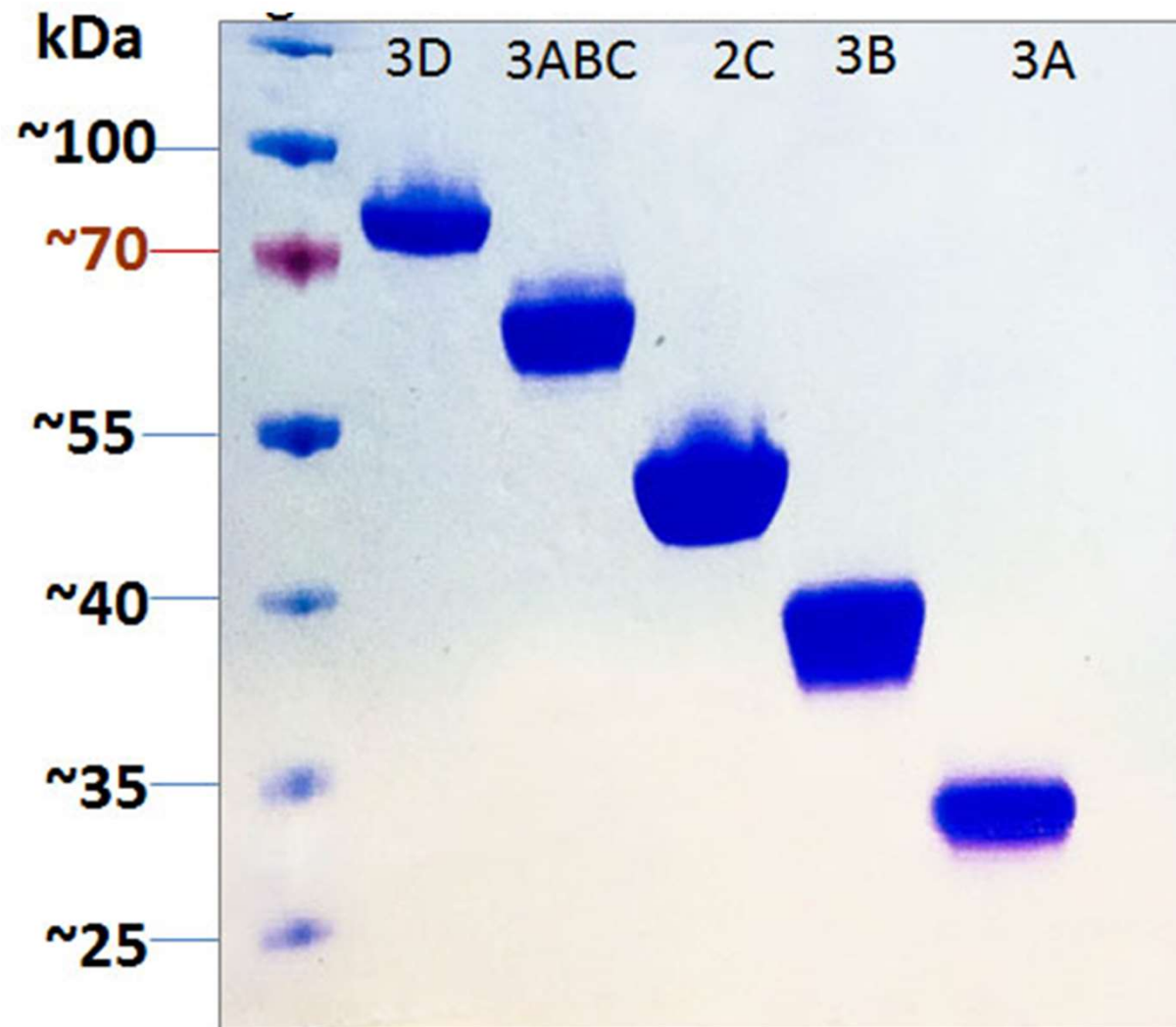
Soro	Tiras Testadas	Resultado				
		3A	3B	2C	3D	3ABC
		()	()	()	()	()
CN		(+)	(+)	(+)	(+)	(+)
CPI		(+)	(+)	(+)	(+)	(+)
CP2		(+)	(+)	(+)	(+)	(+)
		()	()	()	()	()
(PAINELO OTC)		()	()	()	()	()
26		(+)	(+)	(+)	(+)	(+)
27		(+)	(+)	(+)	(+)	(+)
28		(+)	(+)	(+)	(+)	(+)
30		(+)	(+)	(+)	(+)	(+)
31		(+)	(+)	(+)	(+)	(+)
32		(+)	(+)	(+)	(+)	(+)
34		(+)	(+)	(+)	(+)	(+)
35		(+)	(+)	(+)	(+)	(+)
		()	()	()	()	()

EITB V2.0

New clones produced at Panaftosa, 3D 3ABC, 2C, 3B y 3A engineered to improve specificity and easy reading and interpretation



EITB V2.0



3D (73 kDa)

3ABC (55 kDa)

2C (45 kDa)

3B (35 kDa)

3A (25 kDa)



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Panamericana
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Organización
Mundial de la Salud

OFICINA REGIONAL PARA LAS Américas

PANAFTOSA

Centro Panamericano de Fiebre Aftosa
Salud Pública Veterinaria



PANAFTOSA / SPV
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PILOTO EITB19

Caja 2

FA - Prueba Tamiz (3ABC) - Caja 1

Prueba Confirmatoria (EITB) - Caja 2

1 Kit = 440 Pruebas Tamiz e 110 Pruebas Confirmatorias

Kit para detección de anticuerpos anti-3ABC, 3D, 2C, 3B y 3A (VFA),
a través de la técnica inmunoenzimática de western blot.

COMPONENTES

CANTIDAD

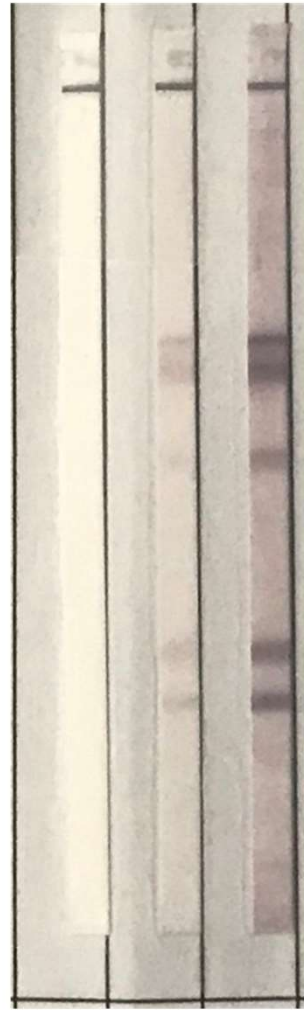
BCIP	1 (500 µL)
Buffer de Lavado 10X	3 (200 mL)
Bula	1 (unidad)
Conjugado	1 (1,2 mL)
Control Negativo	1 (150 µL)
Control Positivo 1	1 (150 µL)
Control Positivo 2	1 (150 µL)
Diluyente de Sustrato	1 (130 mL)
E.coli	1 (250 µL)
Leche en Polvo	1 (25g)
NBT	1 (900 µL)
Tiras Sensibilizadas	125 (5 geles)

Uso sólo para diagnóstico. Mantener refrigerado de 2 a 8 ° C.
Antes de comenzar la prueba, lea las instrucciones adjuntas.

Fab: 03/19
Venc: 03/20
Lote: FAP0119111

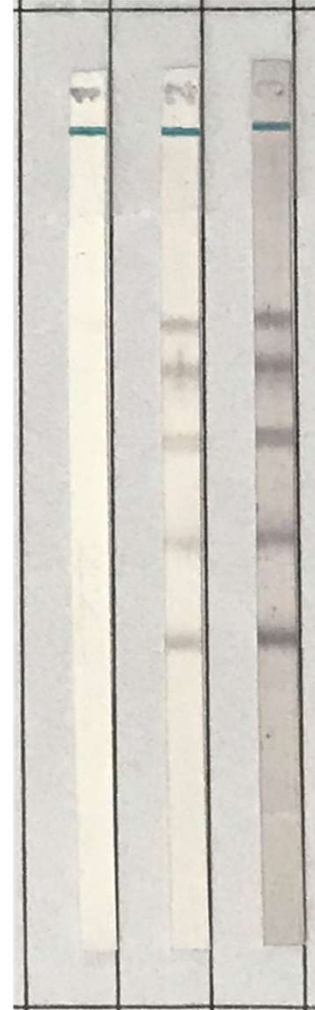
Comparative performance EITB V2.0

CN CP1 CP2



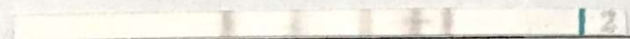
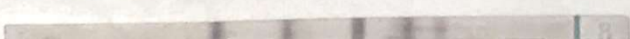
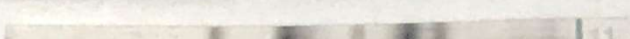
**3ABC
3D
2C
3B
3A**

CN CP1 CP2



**3D
3ABC
2C
3B
3A**

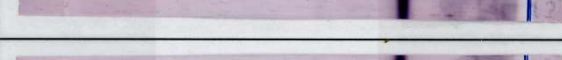
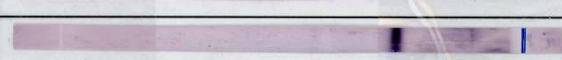
Overall results EITB V2.0

Soro	Tiras Testadas	Resultado				
		3A	3B	2C	3ABC	3D
		()	()	()	()	()
CN		()	()	()	()	()
CPL		()	()	()	()	()
CP2		()	()	()	()	()
		()	()	()	()	()
(PAINEL OIE)		()	()	()	()	()
26		(+)	(+)	(+)	(+)	(+)
27		(+)	(+)	(+)	(+)	(+)
28		(+)	(+)	(+)	(+)	(+)
30		(+)	(+)	(+)	(+)	(+)
31		(+)	(+)	(+)	(+)	(+)
32		(+)	(+)	(+)	(+)	(+)
34		(+)	(+)	(+)	(+)	(+)
35		(+)	(+)	(+)	(+)	(+)
		()	()	()	()	()

EITB V2.0 in experimentally infected cattle

Serum	Strip test	Result					Result
		3A	3B	2C	3ABC	3D	
C145-DPI 0		(-)	(-)	(-)	(-)	(-)	Negative
DPI 1		(-)	(-)	(-)	(-)	(-)	Negative
DPI 2		(-)	(-)	(-)	(-)	(-)	Negative
DPI 3		(-)	(-)	(-)	(-)	(-)	Negative
DPI 4		(-)	(-)	(-)	(-)	(-)	Negative
DPI 5		(-)	(-)	(-)	(-)	(-)	Negative
DPI 7		(-)	(+)	(-)	(+)	(+)	positive
DPI 10		(+)	(+)	(+)	(+)	(+)	positive
DPI 14		(+)	(+)	(+)	(+)	(+)	positive
DPI 17		(+)	(+)	(+)	(+)	(+)	positive
DPI 22		(+)	(+)	(+)	(+)	(+)	positive
DPI 24		(+)	(+)	(+)	(+)	(+)	positive
Pos.1		(+)	(+)	(+)	(+)	(+)	} controls ok.
Pos.2		(+)	(+)	(+)	(+)	(+)	
Neg.		(-)	(-)	(-)	(-)	(-)	

EITB V2.0 in experimentally infected cattle

		3A 3B 2C 3ABC 3D				
10	C146-0		(-)	(-)	(-)	(-)
11	1		(-)	(-)	(-)	(-)
12	2		(-)	(-)	(-)	(-)
13	3		(-)	(-)	(-)	(-)
14	4		(-)	(-)	(-)	(-)
15	5		(-)	(-)	(-)	(-)
16	7		(+)	(+)	(+)	(+)
17	9		(+)	(+)	(+)	(+)
18	11		(+)	(+)	(+)	(+)
19	15		(+)	(+)	(+)	(+)
20	17		(+)	(+)	(+)	(+)
21	22		(+)	(+)	(+)	(+)
22	25		(+)	(+)	(+)	(+)
23	Pos.1		(+)	(+)	(+)	(+)
24	Pos.2		(+)	(+)	(+)	(+)
25	Neg.		(-)	(-)	(-)	(-)

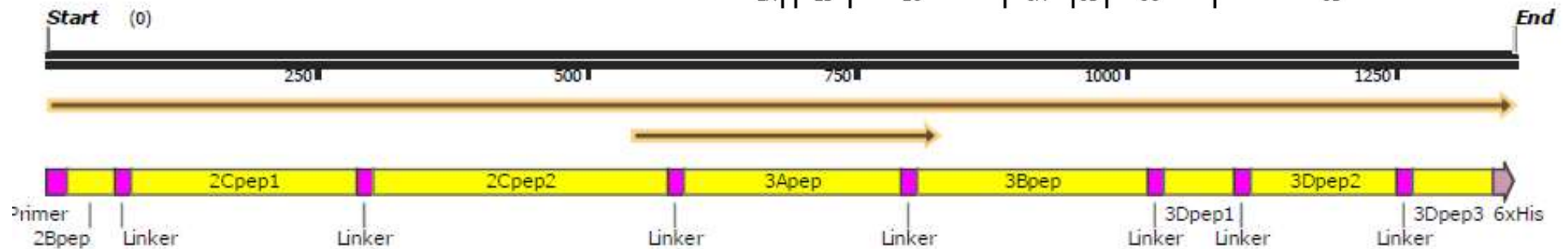
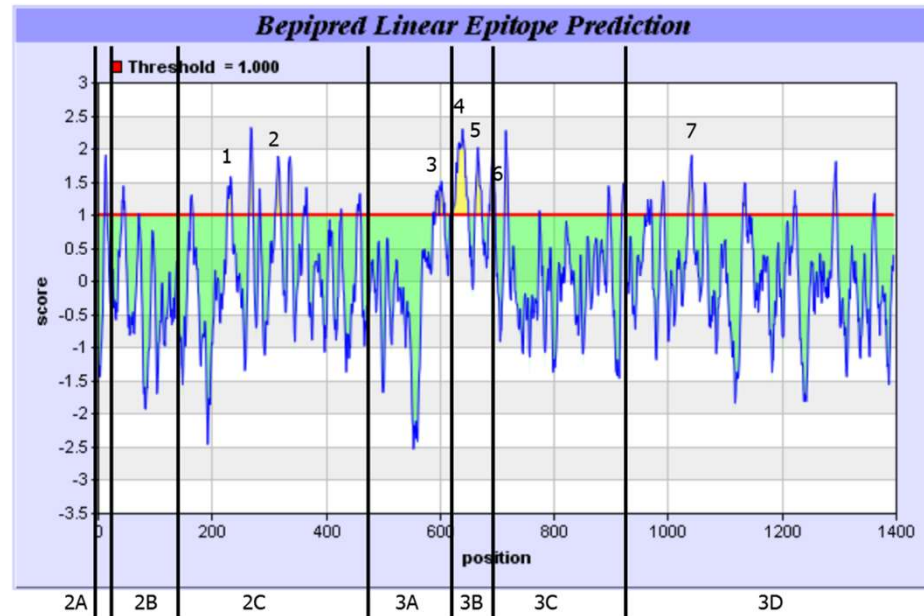
EITB V2.0 in experimentally infected sheep

Serum	Strip test	Result				
		3A	3B	2C	3ABC	3D
S656-DPI0		(+)	(-)	(-)	(-)	(-)
1		(+)	(-)	(-)	(-)	(-)
2		(+)	(-)	(-)	(-)	(-)
3		(+)	(-)	(-)	(-)	(-)
5		(+)	(-)	(-)	(-)	(+)
6		(+)	(-)	(-)	(-)	(+)
7		(+)	(+)	(+)	(+)	(+)
8		(+)	(+)	(+)	(+)	(+)
9		(+)	(+)	(+)	(+)	(+)
13		(+)	(+)	(+)	(+)	(+)
20		(+)	(+)	(+)	(+)	(+)
30		(+)	(+)	(+)	(+)	(+)
S804-DPI0		(-)	(-)	(-)	(-)	(-)
1		(-)	(-)	(-)	(-)	(+)
2		(-)	(-)	(-)	(-)	(+)
3		(-)	(-)	(-)	(-)	(+)
4		(-)	(-)	(-)	(-)	(+)
6		(+)	(+)	(+)	(+)	(+)
7		(+)	(+)	(+)	(+)	(+)
8		(+)	(+)	(+)	(+)	(+)
9		(+)	(+)	(+)	(+)	(+)
Pos.1		(+)	(+)	(+)	(+)	(+)
Pos.2		(+)	(+)	(+)	(+)	(+)
Neg.		(-)	(-)	(-)	(-)	(-)

Conclusions and future direction

- Panaftosa has developed a new version of the 3ABC ELISA (blocking).
- The bELISA has an increases specificity when compared to the previous version of the Panaftosa 3ABC ELISA or iELISA.
- The bELISA can be used to test any ruminant species.
- bELISA in pigs has lower sensitivity and alternative ELISA formats/antigens needs to be evaluated.
- Additional validation is required before the kit becomes fully available (vaccinated and naïve South American populations).

Multiepitope antigen ELISA for the deletion of NSP antibodies in Swine



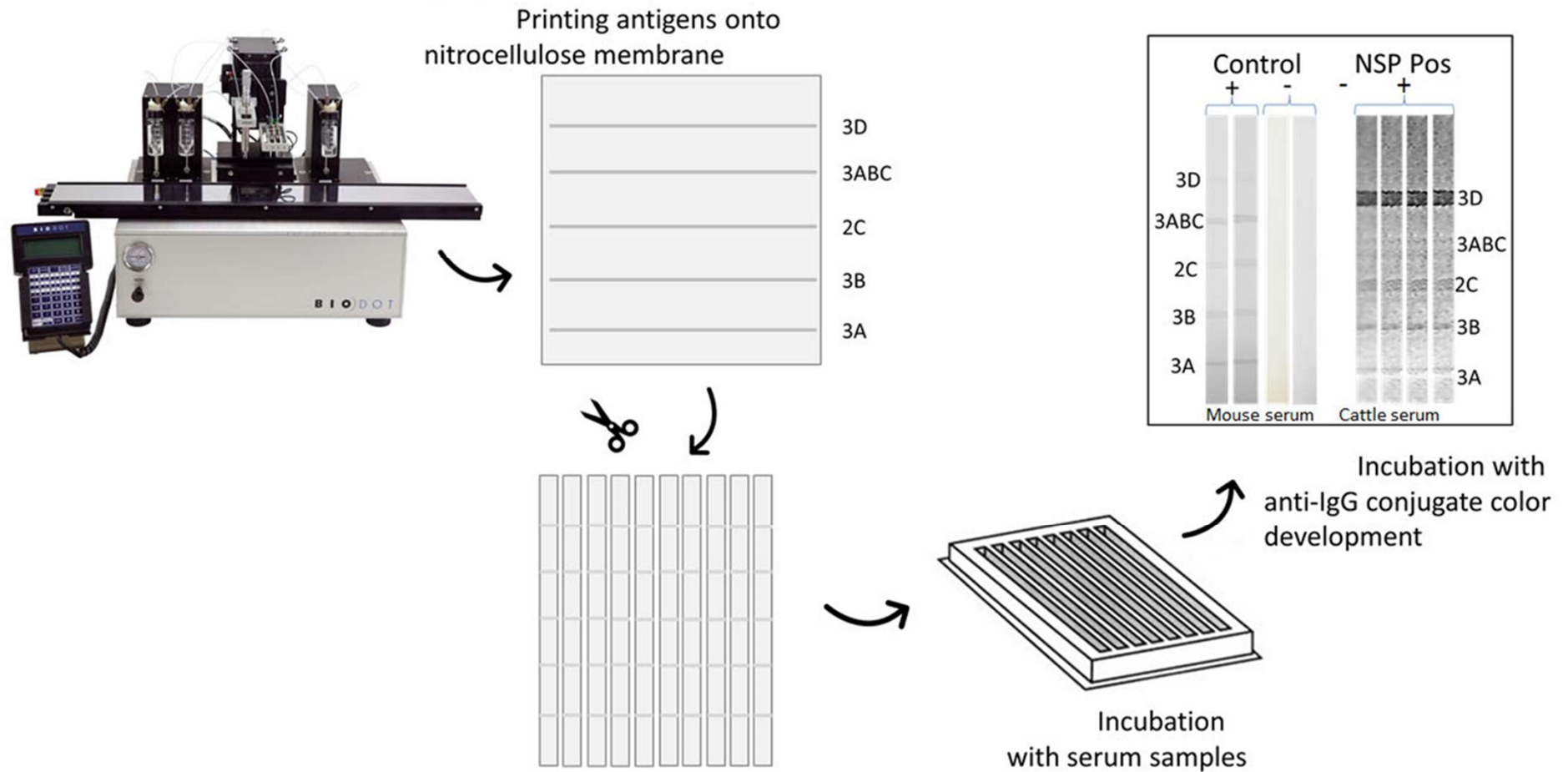
Performance of NSP4 iELISA in swine

	3ABC cELISA	NSP4 iELISA			3ABC cELISA	NSP4 iELISA			3ABC cELISA	NSP4 iELISA	
Sample	%Inhib	OD	%Inhib	Sample	%Inhib	OD	%Inhib	Sample	%Inhib	OD	%Inhib
P3 (dpi -1)	2.25	0.09	2.81	P10 (dpi -1)	-69.89	0.10	3.17	P13 (dpi -1)	-56.85	0.09	2.86
P3 (dpi 1)	-6.71	0.08	2.33	P10 (dpi 1)	-75.00	0.12	3.63	P13 (dpi 1)	-42.88	0.06	1.90
P3 (dpi 2)	2.21	0.09	2.77	P10 (dpi 2)	-72.54	0.12	3.81	P13 (dpi 2)	-40.64	0.08	2.61
P3 (dpi 3)	-1.64	0.08	2.42	P10 (dpi 3)	-78.17	0.09	2.78	P13 (dpi 3)	-49.09	0.09	2.68
P3 (dpi 4)	-28.29	0.07	2.26	P10 (dpi 4)	-75.92	0.08	2.58	P13 (dpi 4)	-82.06	0.09	2.74
P3 (dpi 5)	-24.26	0.08	2.43	P10 (dpi 5)	-73.25	0.10	3.14	P13 (dpi 5)	-46.68	0.11	3.52
P3 (dpi 6)	-14.99	0.08	2.63	P10 (dpi 6)	-68.50	0.11	3.39	P13 (dpi 6)	-47.61	0.16	4.99
P3 (dpi 7)	4.50	0.20	6.14	P10 (dpi 7)	-74.94	0.12	3.58	P13 (dpi 7)	-26.94	0.33	10.24
P3 (dpi 14)	46.64	0.53	16.46	P10 (dpi 14)	-50.64	3.65	113.19	P13 (dpi 14)	58.62	3.12	96.80
P3 (dpi 21)	50.15	0.86	26.53	P10 (dpi 21)	-29.19	3.35	103.97	P13 (dpi 21)	63.25	3.13	96.91
P3 (dpi 28)	81.29	1.60	49.70	P10 (dpi 28)	-17.92	3.59	111.14	P13 (dpi 28)	65.08	2.03	63.04
Sample	%Inhib	OD	%Inhib	Sample	%Inhib	OD	%Inhib	Sample	%Inhib	OD	%Inhib
P5 (dpi -1)	-42.39	0.10	3.04	P12 (dpi -1)	-37.08	0.11	3.36	P17 (dpi -1)	-91.88	0.18	5.61
P5 (dpi 1)	-49.67	0.08	2.37	P12 (dpi 1)	-37.84	0.10	3.19	P17 (dpi 1)	-77.10	0.14	4.42
P5 (dpi 2)	-36.64	0.08	2.55	P12 (dpi 2)	-33.50	0.09	2.92	P17 (dpi 2)	-65.73	0.13	4.03
P5 (dpi 3)	-37.21	0.09	2.71	P12 (dpi 3)	-35.38	0.10	3.23	P17 (dpi 3)	-105.91	0.12	3.60
P5 (dpi 4)	-45.58	0.09	2.86	P12 (dpi 4)	-37.36	0.11	3.44	P17 (dpi 4)	-107.93	0.13	3.88
P5 (dpi 5)	-47.09	0.07	2.23	P12 (dpi 5)	-36.35	0.12	3.74	P17 (dpi 5)	-104.76	0.12	3.63
P5 (dpi 6)	-53.98	0.08	2.41	P12 (dpi 6)	-31.55	0.10	3.06	P17 (dpi 6)	-98.16	0.13	3.95
P5 (dpi 7)	-33.33	0.09	2.74	P12 (dpi 7)	-16.95	0.15	4.65	P17 (dpi 7)	-97.29	0.14	4.30
P5 (dpi 14)	54.92	3.23	100.00	P12 (dpi 14)	40.80	1.65	51.12	P17 (dpi 14)	-74.74	1.46	45.37
P5 (dpi 21)	54.97	2.78	86.21	P12 (dpi 21)	82.46	3.91	121.29	P17 (dpi 21)	-4.20	2.12	65.73
				P12 (dpi 28)	79.70	3.69	114.34	P17 (dpi 28)	-14.27	1.42	43.91

Conclusions and future direction

- New recombinant proteins were developed and used in the EITB (EITB V2.0).
- The EITB V2.0 facilitate reading and interpretation.
- Further optimization and validation is required before EITB kits become available.

EITB V3.0 Multi-antigen Print Immunoassay (MAPIA)



Canada

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