

Nueva generación de kits para el diagnóstico de Fiebre Aftosa.

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COSALFA 46
Mayo 3, 2019

INFORME FINAL DEL SEMINARIO INTERNACIONAL PRE COSALFA 2020 EN EL HORIZONTE: DESAFÍOS PARA EL PHEFA

*Santa Cruz de la Sierra, Bolivia | 16 y 17 de
abril de 2018*



1. Se evidencia que las pruebas diagnósticas deben evolucionar, incorporar cambios, adaptadas para dar respuesta al proceso de transición epidemiológico de los países de la región.
2. Se entiende que, en el marco del status epidemiológico regional, la detección rápida y caracterización de cepas son objetivos prioritarios.
3. Pruebas con alta especificidad son ahora requeridas para disminuir la tasa de falsos positivos, e incrementar la posibilidad de asegurar la detección de animales positivos.

MATERIAL TRANSFER AND DATA SHARING AGREEMENT

This agreement made this 27th day of February, 2018 (the "Agreement") by and between the Canadian Food Inspection Agency, a body corporate established pursuant to the Canadian Food Inspection Act, SC 1997, c.6, having its headquarters located at 1400 Merivale Road, Ottawa, Ontario, Canada, K1A 0Y9 (hereinafter referred to as "Provider")

AND

The Pan American Health Organization ("PAHO"), through its Pan American Foot-and-Mouth Disease Center ("PANAFMOSA"), having its headquarters located at Av. Governador Leonel de Moura Brizola (antigua Av. Pres. Kennedy) 7778, Sao Bento (CEP 25045-002), Duque de Caxias, Rio de Janeiro, Brazil (hereinafter referred to as "Recipient")

Collectively referred to as the "Parties".

The Parties agree to as follows:

1. Definitions:

"Progeny": Unmodified material originated from the Material (as defined below) such as virus from virus or cell from cell.

"Unmodified Derivatives": substances created by the Recipient which constitute an unmodified functional subunit or product originated from the Material.

"Material": material being transferred according to Section 2 a) and b) hereof.

"Data": data being transferred according to Section 3 hereof.

"Modifications": Substances created by the Recipient which contain or incorporate the Material.

2. Material Transfer:

Provider agrees to transfer to the Recipient the following Material:

- a) Bacterial cell clone producing recombinant foot-and-mouth disease virus (FMDV) non-structural protein 3ABC: Escherichia coli cell (BL21 (DE3)) pLysS clone transformed with recombinant Pet30a vector expressing recombinant His-tagged 3ABC protein; and
b) A hybridoma producing monoclonal antibody (Mab) anti-FMDV non-structural protein 3B (Mab F83Bp 1-3)

13.1 This Agreement shall be interpreted in accordance with the laws in force in the Province of Manitoba and the federal laws of Canada applicable therein.

Signed for and on behalf of: the Canadian Food Inspection Agency (Provider)

[Handwritten signature of Dr. Alfonso Clavijo]

Dr. Alfonso Clavijo
Laboratory Executive Director
National Centres for Animal Diseases

Date:

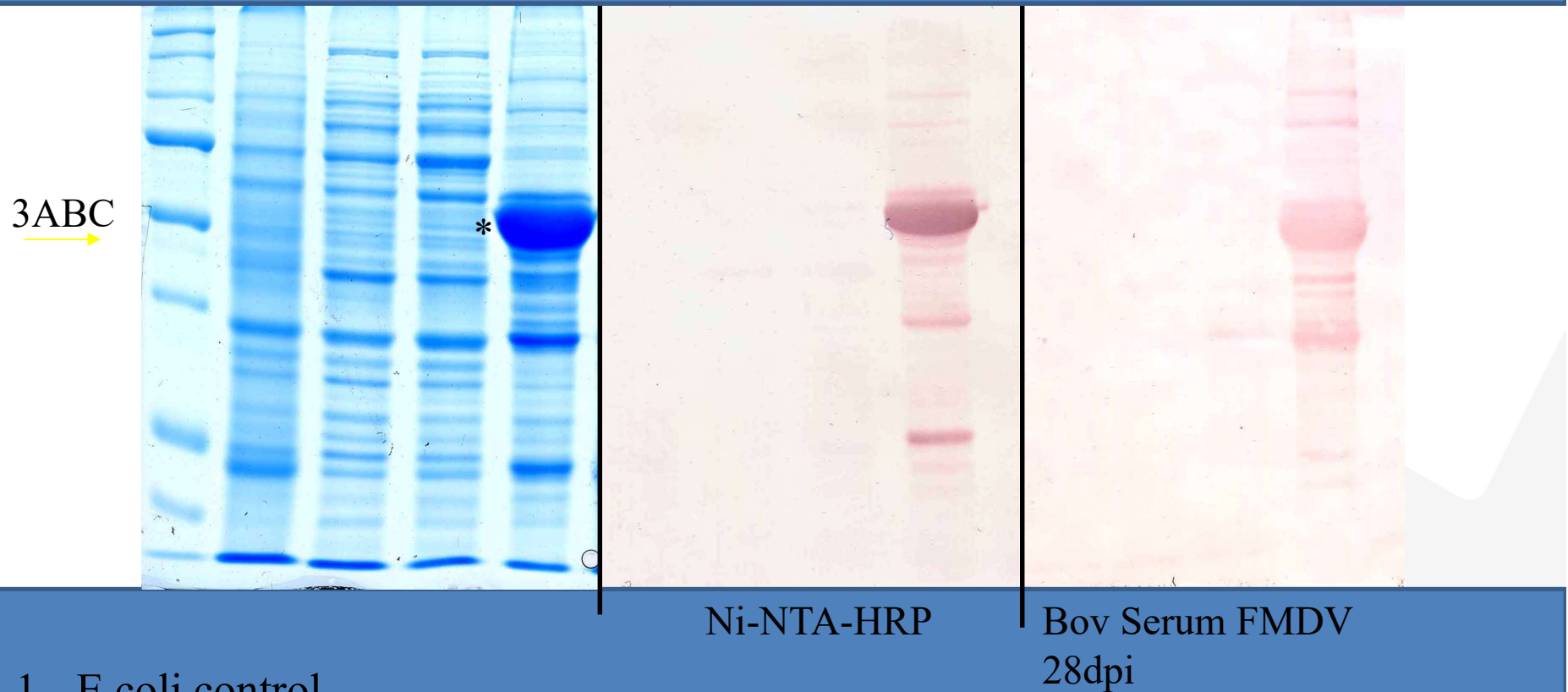
Signed for and on behalf of: the Pan American Health Organization (Recipient)

[Handwritten signature of Carissa F. Etienne]

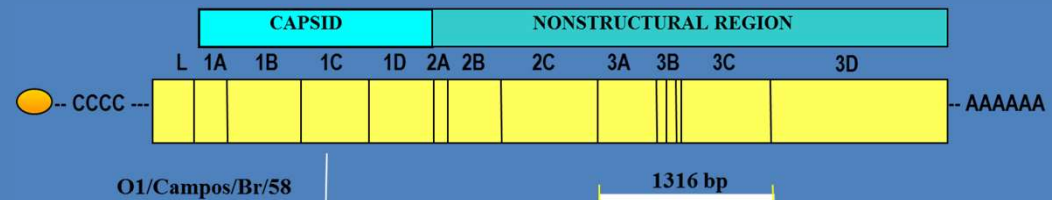
Carissa F. Etienne
Director

Date: 27 February 2018

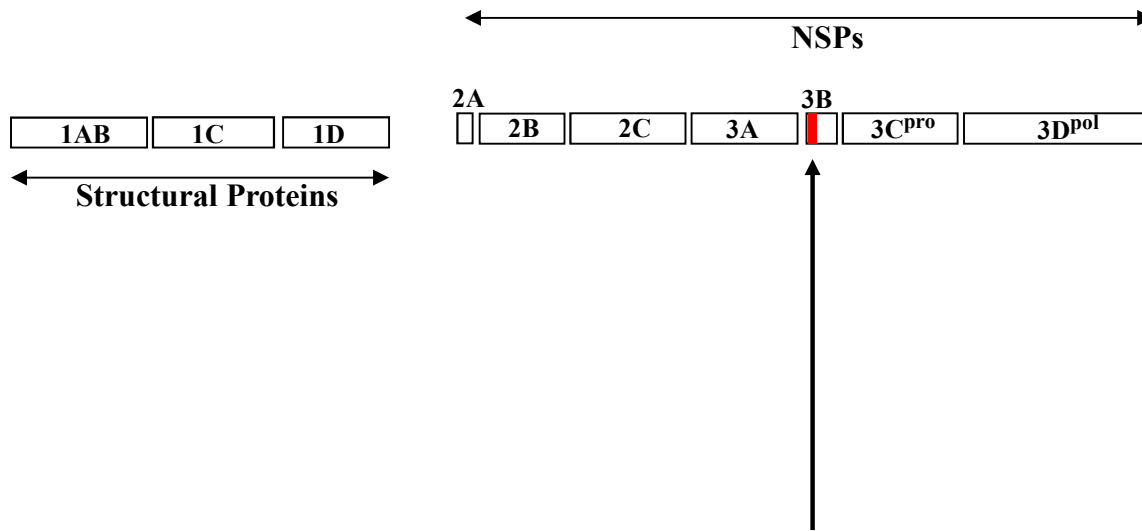
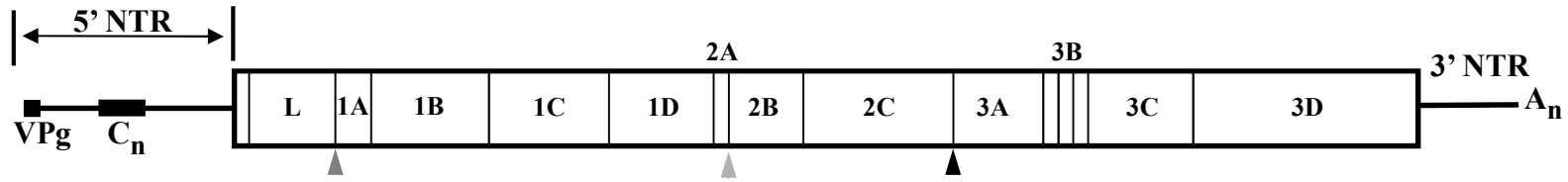
Recombinant protein 3ABC



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



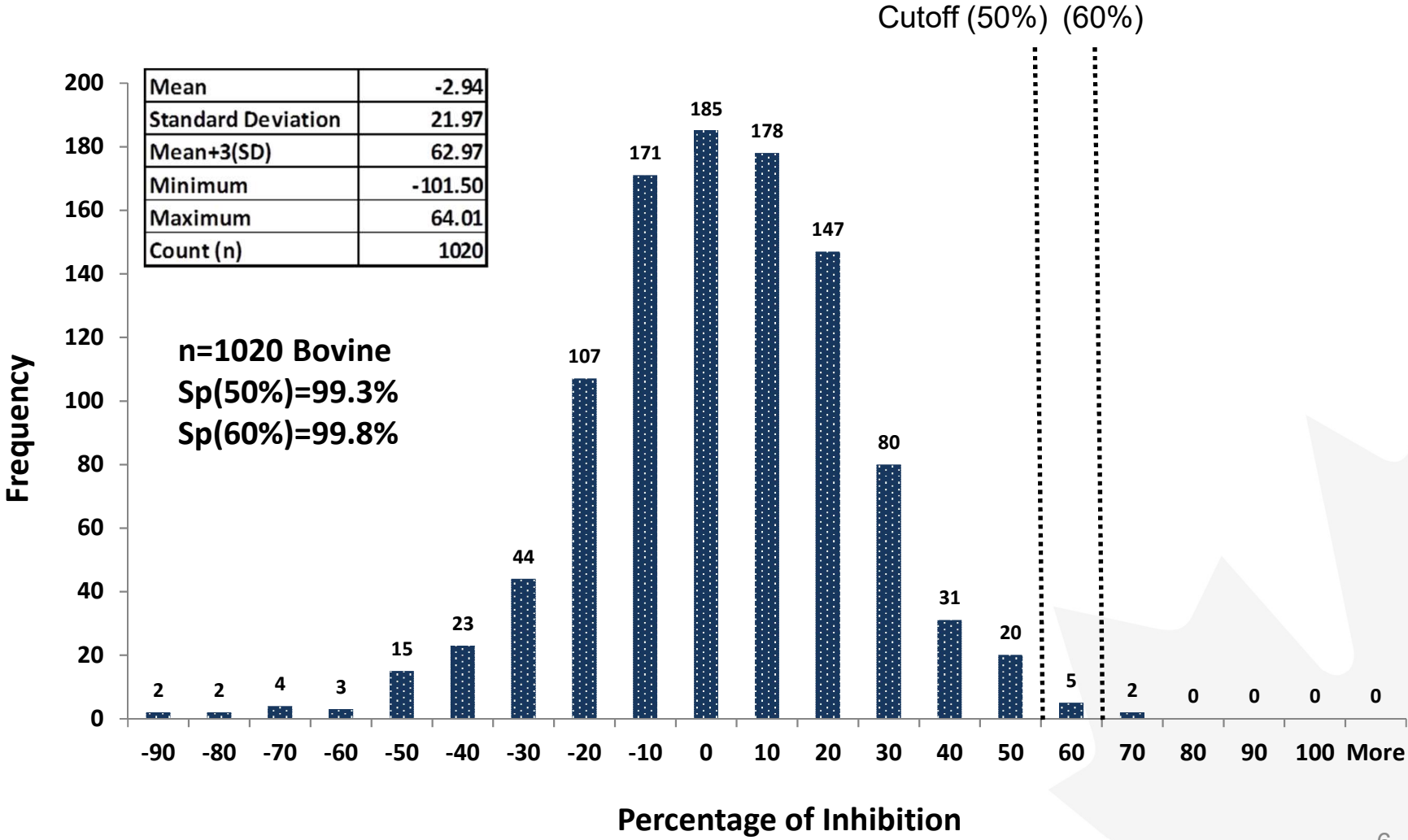
October 30, 2017



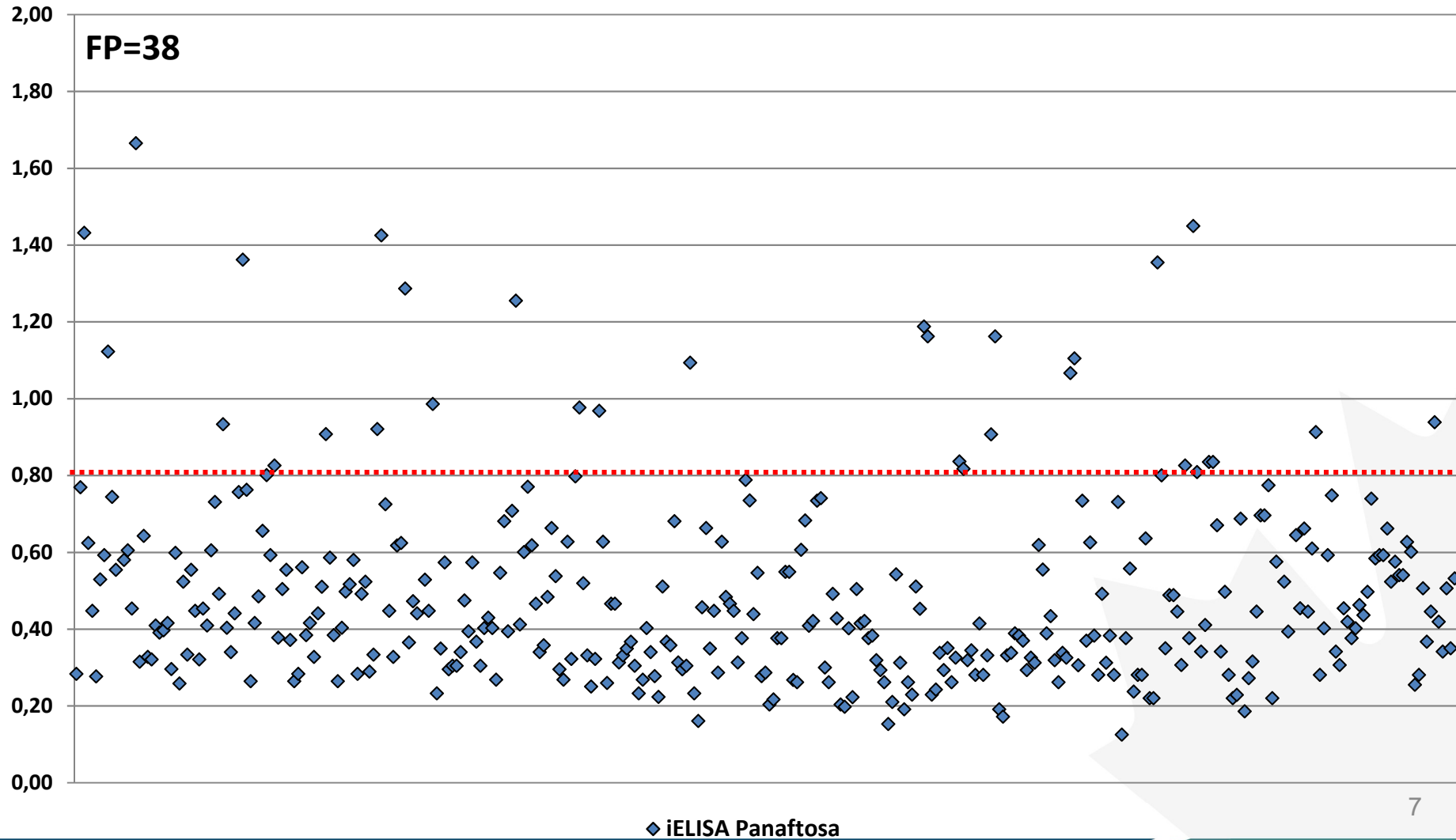
NH₂-CGPYAGPLERQKPLK-COOH

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

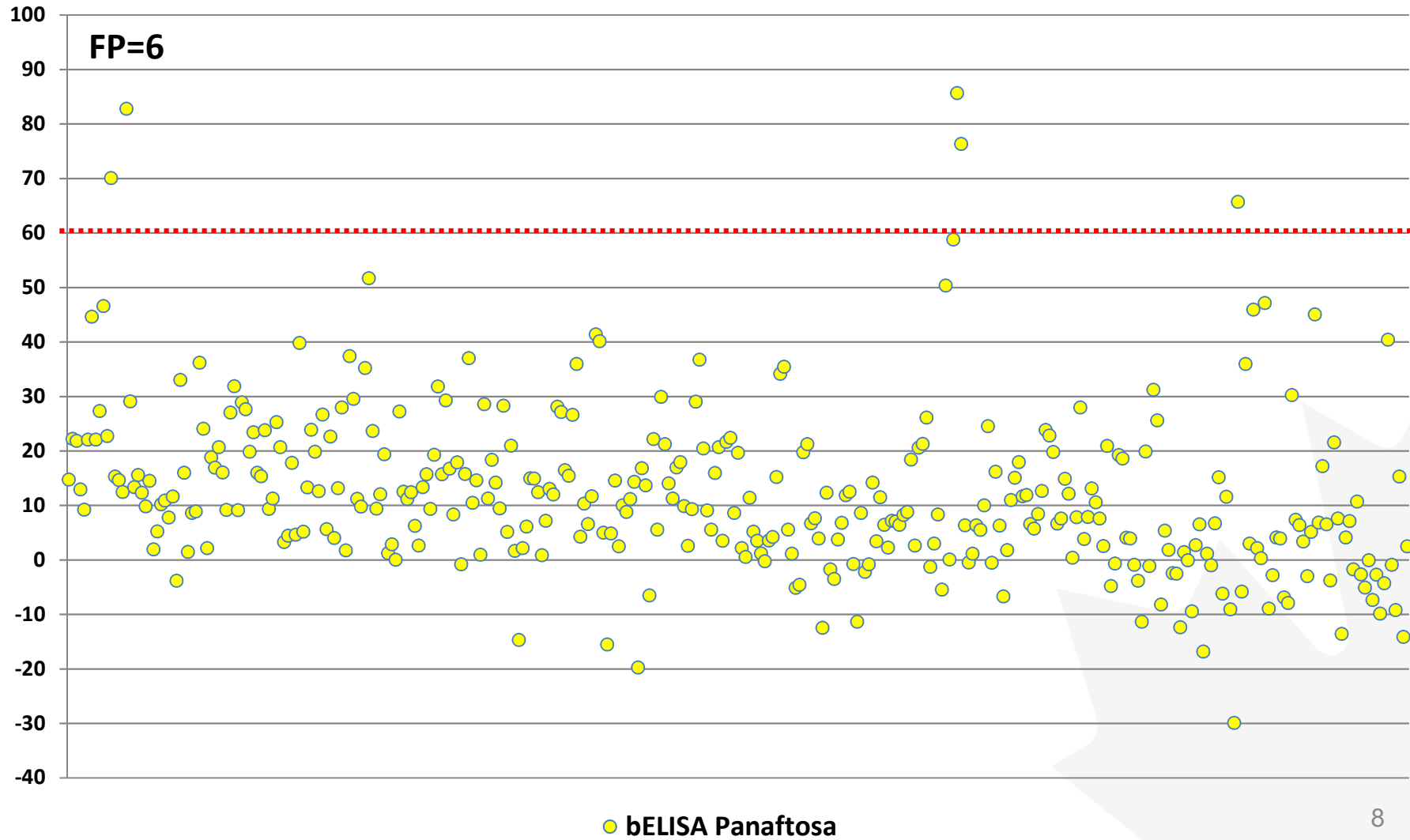
3ABC bELISA distribution of Cattle Negative Samples



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)



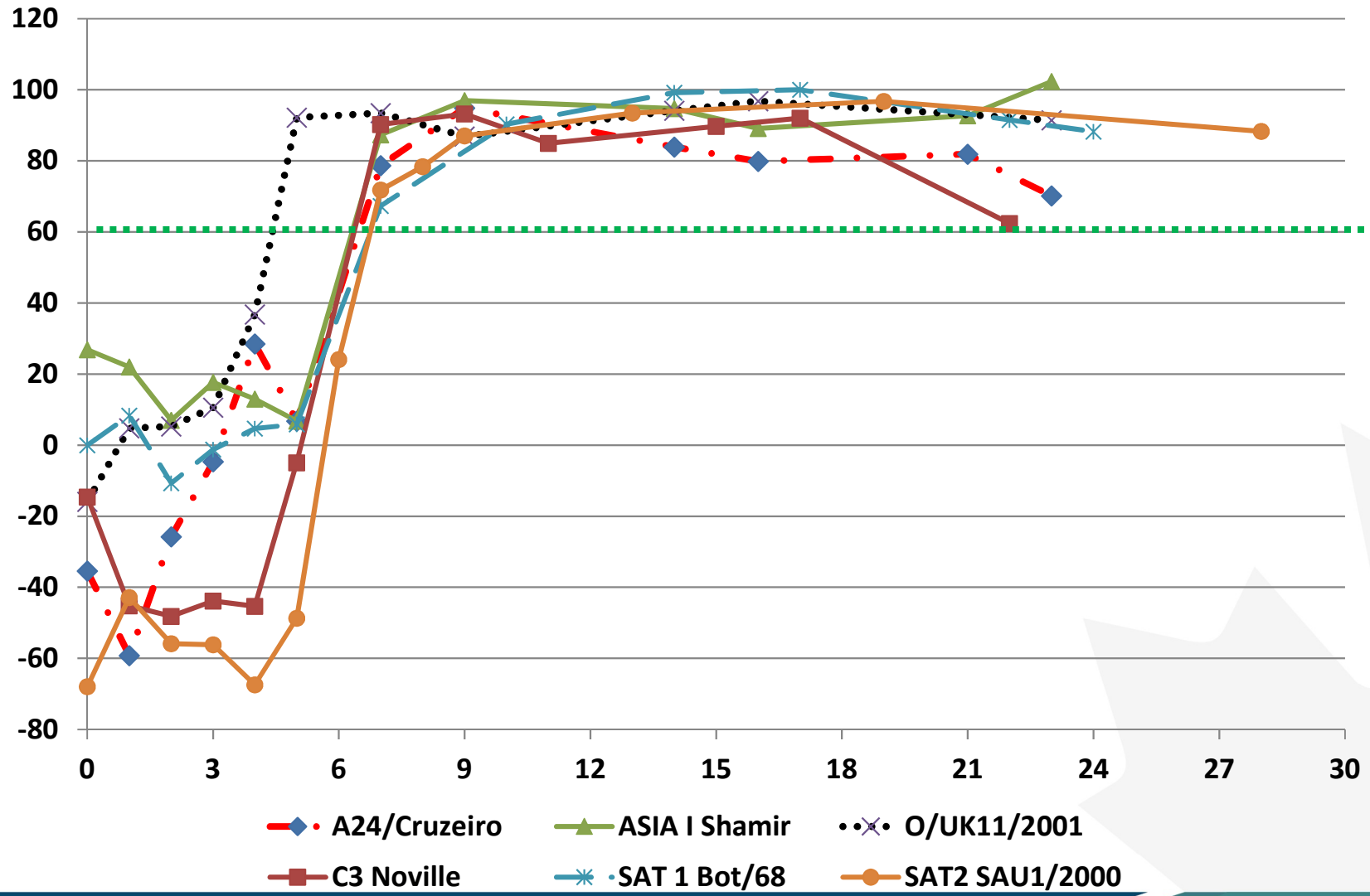
3ABC iELISA Vs bELISA Panaftosa Vaccinated Cattle (13-24; 25-36; >36)

	iELISA			bELISA 60%			
Edad meses	Pos	Neg	DxSp	Pos	Neg	DxSp	Total
13-24	10	92	90.19	2	100	98.03	102
25-36	7	71	91.02	2	76	97.4	78
>36	21	149	87.64	2	168	98.8	170
Total	38	312	89.14	6	344	98.2	350

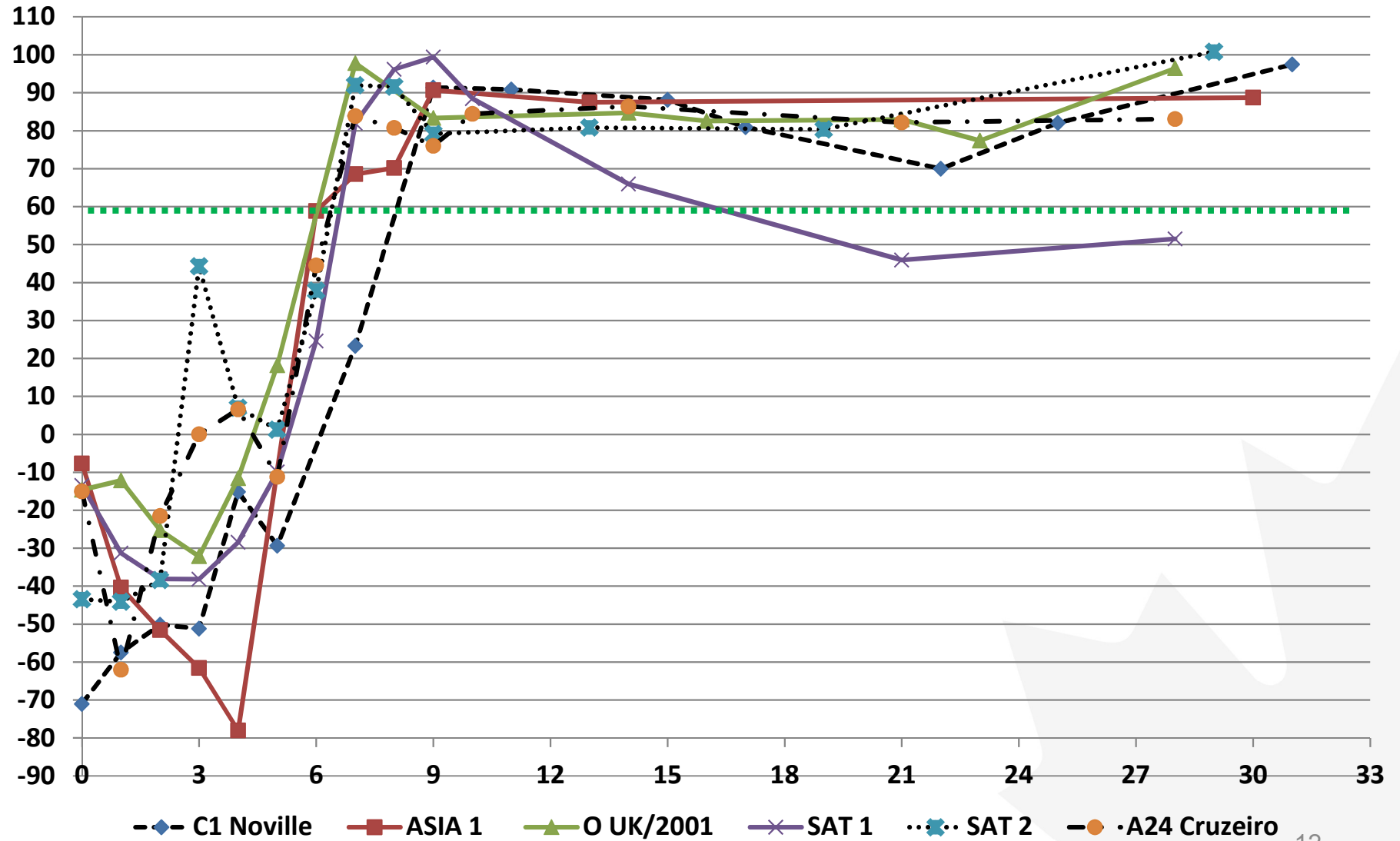
3ABC bELISA experimentally infected Cattle

	PrioCheck	bELISA		PrioCheck	bELISA		PrioCheck	bELISA
A24 Cruzeiro: C140-DPI 0	2.9	-35	OUGK11/2001: C138-DPI 0	18.5	12	Asia 1 Shamir: C134-DPI 0	3.6	-20
A24 Cruzeiro: C140-DPI 1	4.2	-59	OUGK11/2001: C138-DPI 1	28.7	19	Asia 1 Shamir: C134-DPI 1	3.6	-41
A24 Cruzeiro: C140-DPI 2	3.3	-26	OUGK11/2001: C138-DPI 2	14.8	71	Asia 1 Shamir: C134-DPI 2	4.1	-9
A24 Cruzeiro: C140-DPI 3	9.3	-5	OUGK11/2001: C138-DPI 3	10.4	31	Asia 1 Shamir: C134-DPI 3	0.7	-12
A24 Cruzeiro: C140-DPI 4	-3.7	28	OUGK11/2001: C138-DPI 4	18.5	39	Asia 1 Shamir: C134-DPI 4	-6.4	45
A24 Cruzeiro: C140-DPI 5	12.0	7	OUGK11/2001: C138-DPI 5	24.7	71	Asia 1 Shamir: C134-DPI 5	7.4	0
A24 Cruzeiro: C140-DPI 7	44.7	79	OUGK11/2001: C138-DPI 7	70.7	86	Asia 1 Shamir: C134-DPI 7	55.4	72
A24 Cruzeiro: C140-DPI 9	67.5	95	OUGK11/2001: C138-DPI 9	83.4	93	Asia 1 Shamir: C134-DPI 10	92.0	96
A24 Cruzeiro: C140-DPI 14	76.2	84	OUGK11/2001: C138-DPI 14	79.9	82	Asia 1 Shamir: C134-DPI 14	93.2	102
A24 Cruzeiro: C140-DPI 16	77.2	80	OUGK11/2001: C138-DPI 16	77.0	86	Asia 1 Shamir: C134-DPI 17	93.0	101
A24 Cruzeiro: C140-DPI 21	78.5	82	OUGK11/2001: C138-DPI 23	75.6	87	Asia 1 Shamir: C134-DPI 22	92.8	91
A24 Cruzeiro: C140-DPI 23	82.1	70	OUGK11/2001: C138-DPI 28	77.0	87	Asia 1 Shamir: C134-DPI 24	92.3	90
A24 Cruzeiro: C141-DPI 0	-2.5	-34	OUGK11/2001: C139-DPI 1	1.6	-16	Asia 1 Shamir: C145-DPI 0	17.5	27
A24 Cruzeiro: C141-DPI 1	-0.1	-35	OUGK11/2001: C139-DPI 2	-2.8	5	Asia 1 Shamir: C145-DPI 1	23.8	22
A24 Cruzeiro: C141-DPI 2	1.9	-67	OUGK11/2001: C139-DPI 3	-7.0	5	Asia 1 Shamir: C145-DPI 2	21.8	7
A24 Cruzeiro: C141-DPI 3	-3.4	-49	OUGK11/2001: C139-DPI 4	-9.7	11	Asia 1 Shamir: C145-DPI 3	23.4	18
A24 Cruzeiro: C141-DPI 4	0.6	-69	OUGK11/2001: C139-DPI 5	13.1	37	Asia 1 Shamir: C145-DPI 4	24.2	13
A24 Cruzeiro: C141-DPI 5	5.3	-59	OUGK11/2001: C139-DPI 7	46.8	92	Asia 1 Shamir: C145-DPI 5	28.4	7
A24 Cruzeiro: C141-DPI 7	57.6	69	OUGK11/2001: C139-DPI 9	52.7	93	Asia 1 Shamir: C145-DPI 7	61.9	87
A24 Cruzeiro: C141-DPI 9	68.6	85	OUGK11/2001: C139-DPI 14	49.6	87	Asia 1 Shamir: C145-DPI 10	85.5	97
A24 Cruzeiro: C141-DPI 14	59.7	103	OUGK11/2001: C139-DPI 16	60.6	94	Asia 1 Shamir: C145-DPI 14	78.9	95
A24 Cruzeiro: C141-DPI 16	58.6	101	OUGK11/2001: C139-DPI 21	58.4	97	Asia 1 Shamir: C145-DPI 17	76.9	89
A24 Cruzeiro: C141-DPI 21	60.1	96	OUGK11/2001: C139-DPI 23	62.8	91	Asia 1 Shamir: C145-DPI 22	83.3	93
A24 Cruzeiro: C141-DPI 23	64.0	94				Asia 1 Shamir: C145-DPI 24	85.2	102

3ABC bELISA experimentally infected Cattle



3ABC bELISA experimentally infected Sheep



Multiple testing

Parallel testing- Positive in either test =*P*

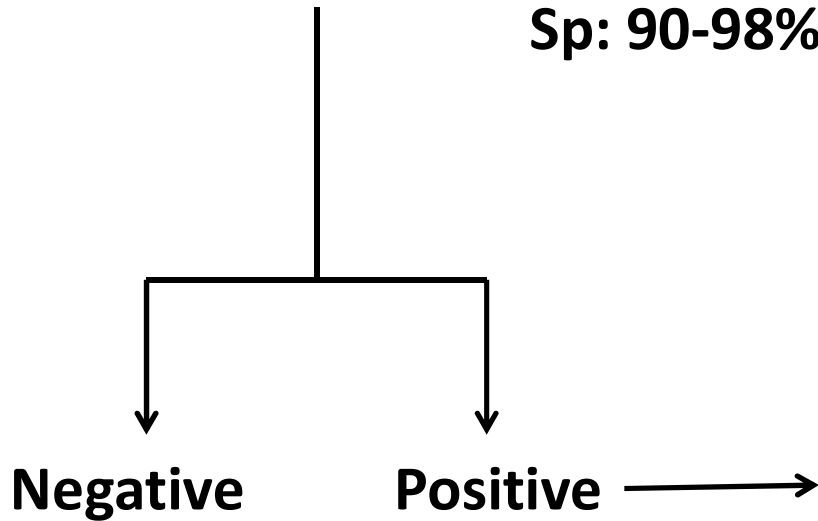
The animal is being asked to “prove” that it is healthy. ↑ Sn and NPV

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%

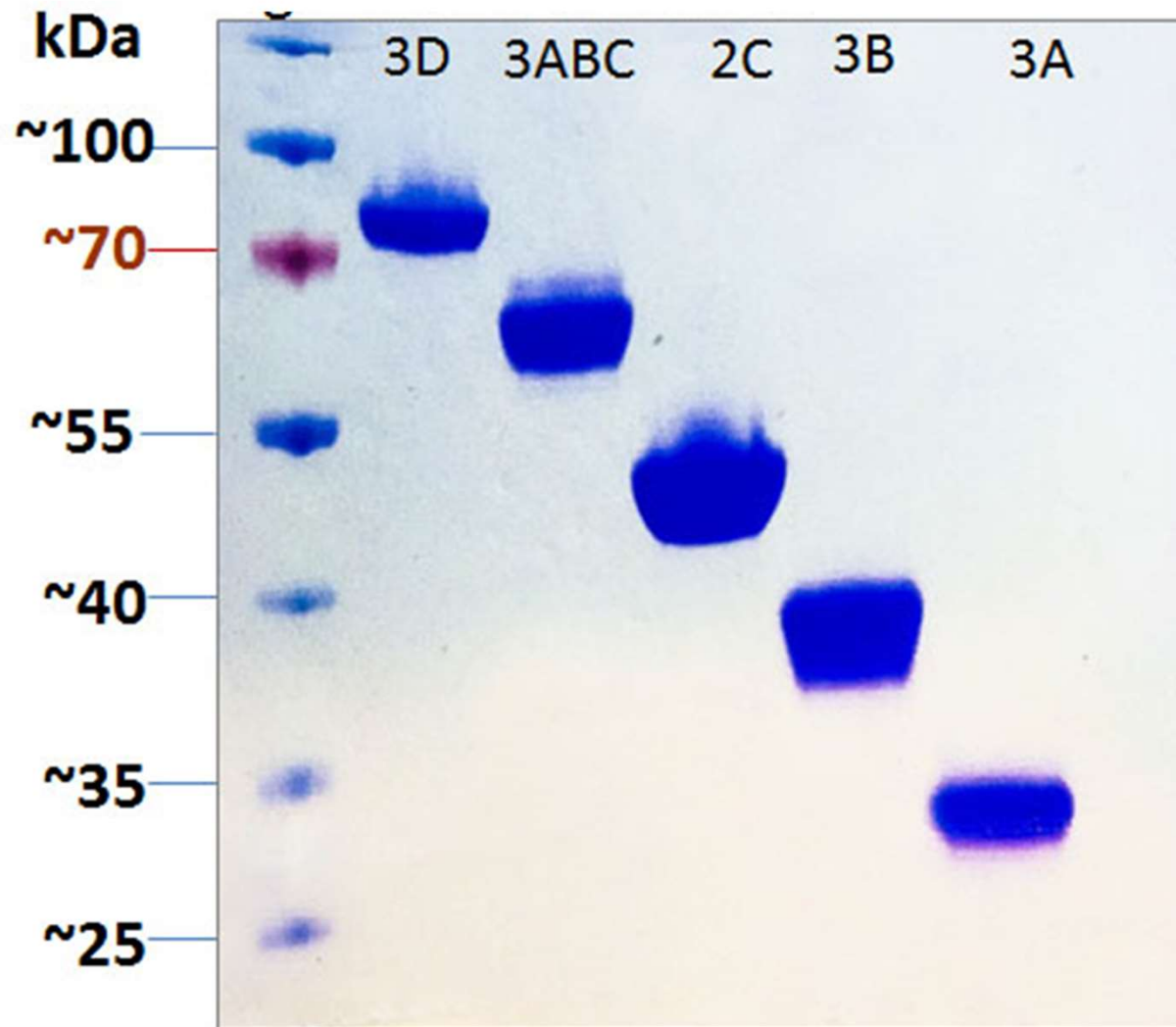


(vaccinated cattle)
Sn: 61% 15-27dpi
23% 28-100 dp

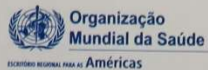
Test with high specificity

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

EITB V2.0



3D (73 kDa)
3ABC (55 kDa)
2C (45 kDa)
3B (35 kDa)
3A (25 kDa)



PANAFTOSA
 Centro Pan-Americano de Febre Aftosa
 Saúde Pública Veterinária



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.

PANAFTOSA / SPV
 OPS / OMS

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)

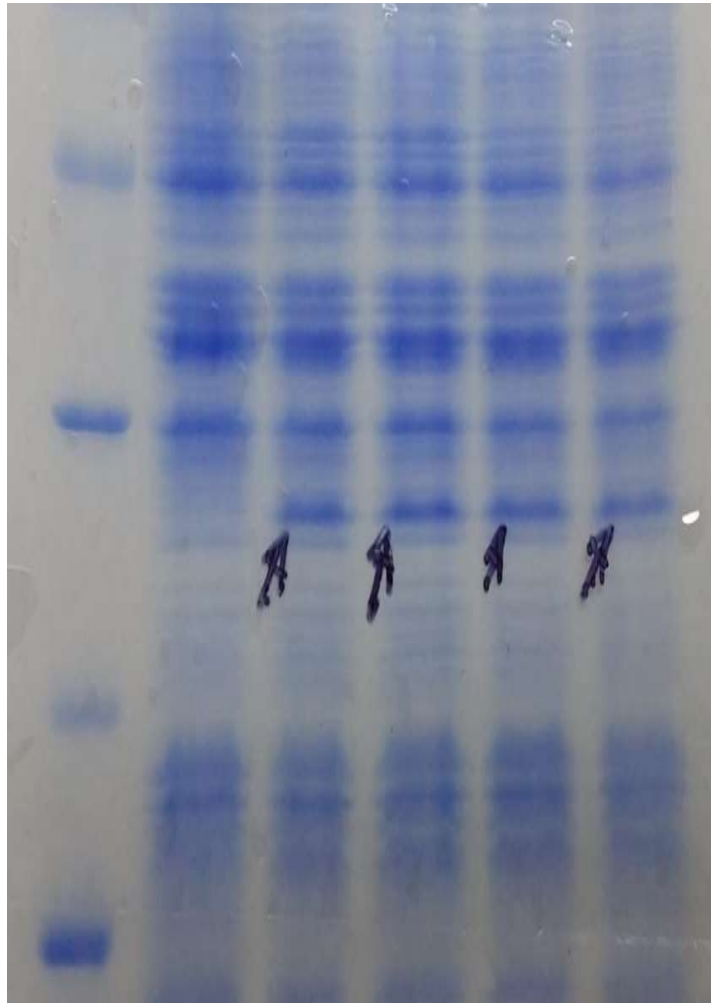
Fab: 03/19
 Venc: 03/20
 Lote: FAP0119111



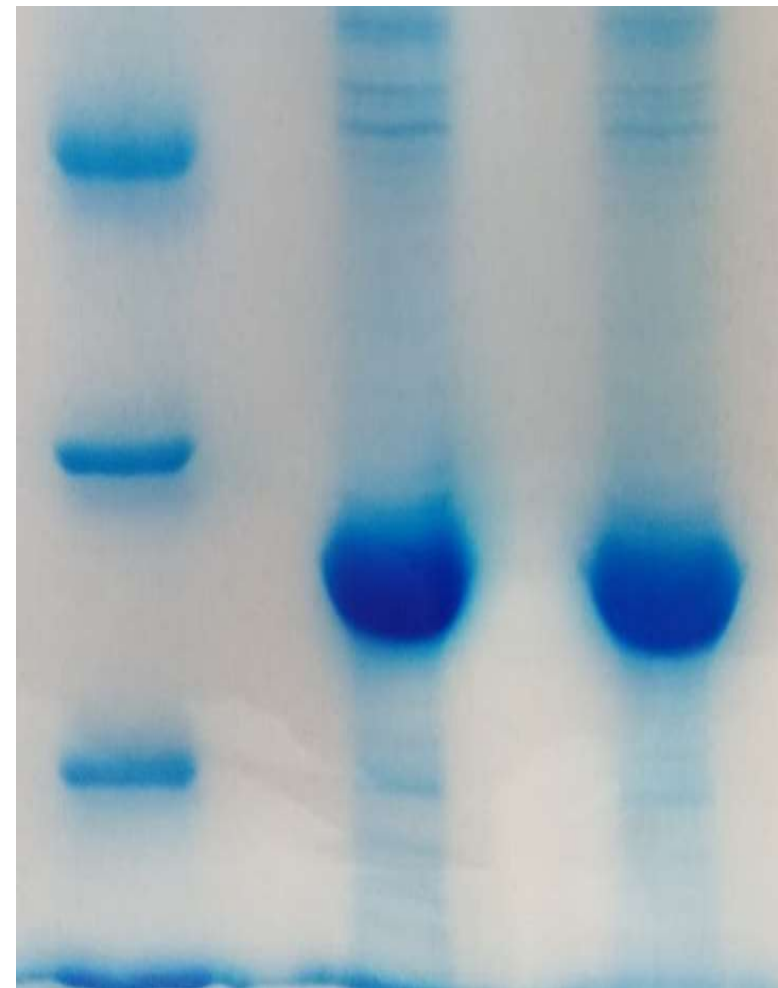
Uso sólo para diagnóstico. Mantener refrigerado de 2 a 8 ° C.
Antes de comenzar la prueba, lea las instrucciones adjuntas.

PRODUCTION AND PURIFICATION OF PROTEINS

Current 3ABC rec protein

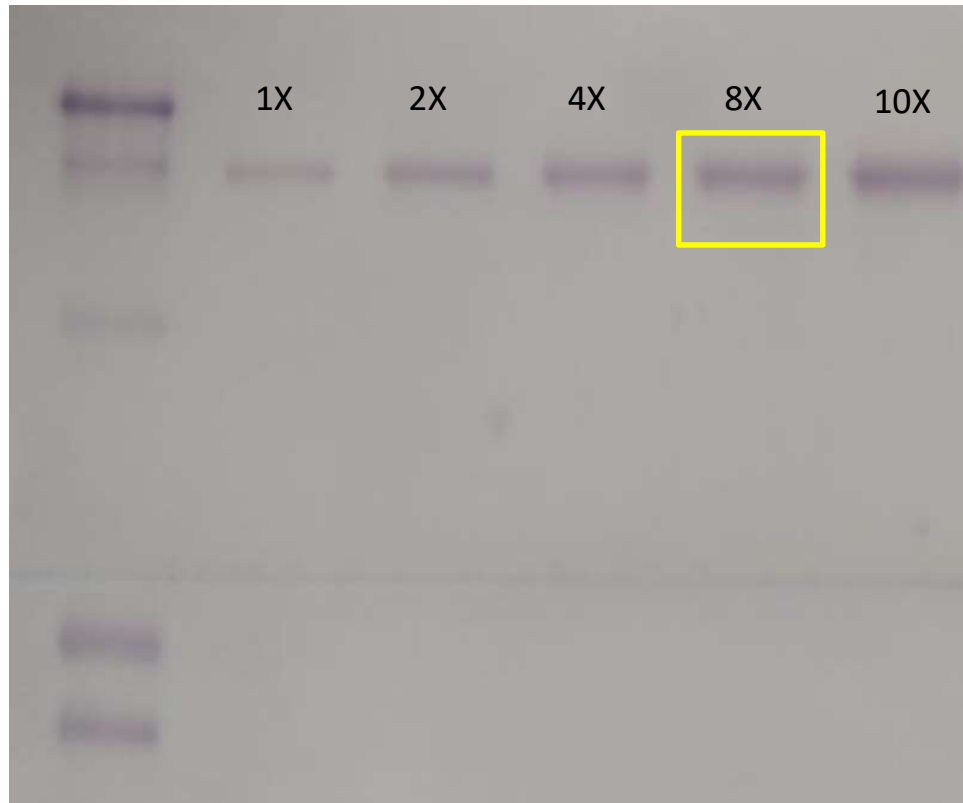


New 3ABC rec protein

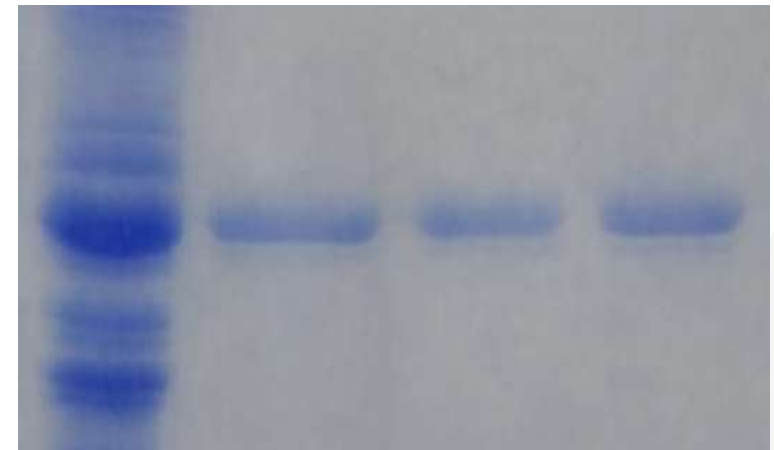


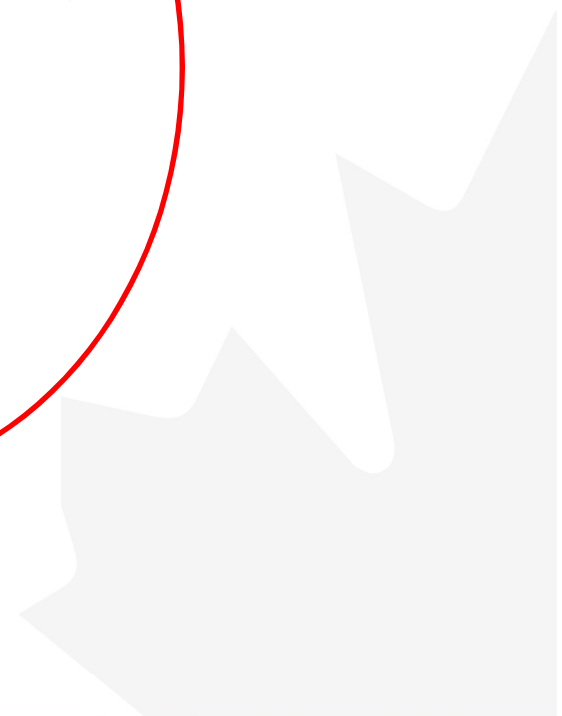
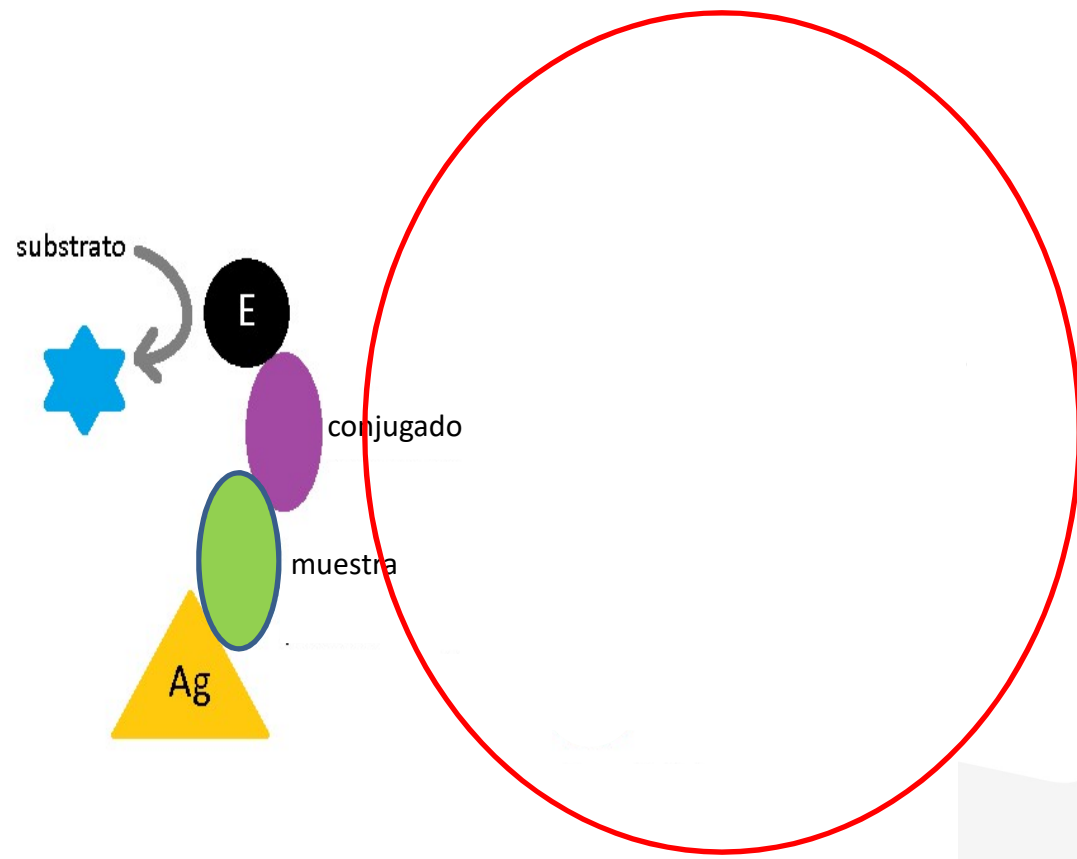
AFTER ELECTROELUTION

Current 3ABC rec protein

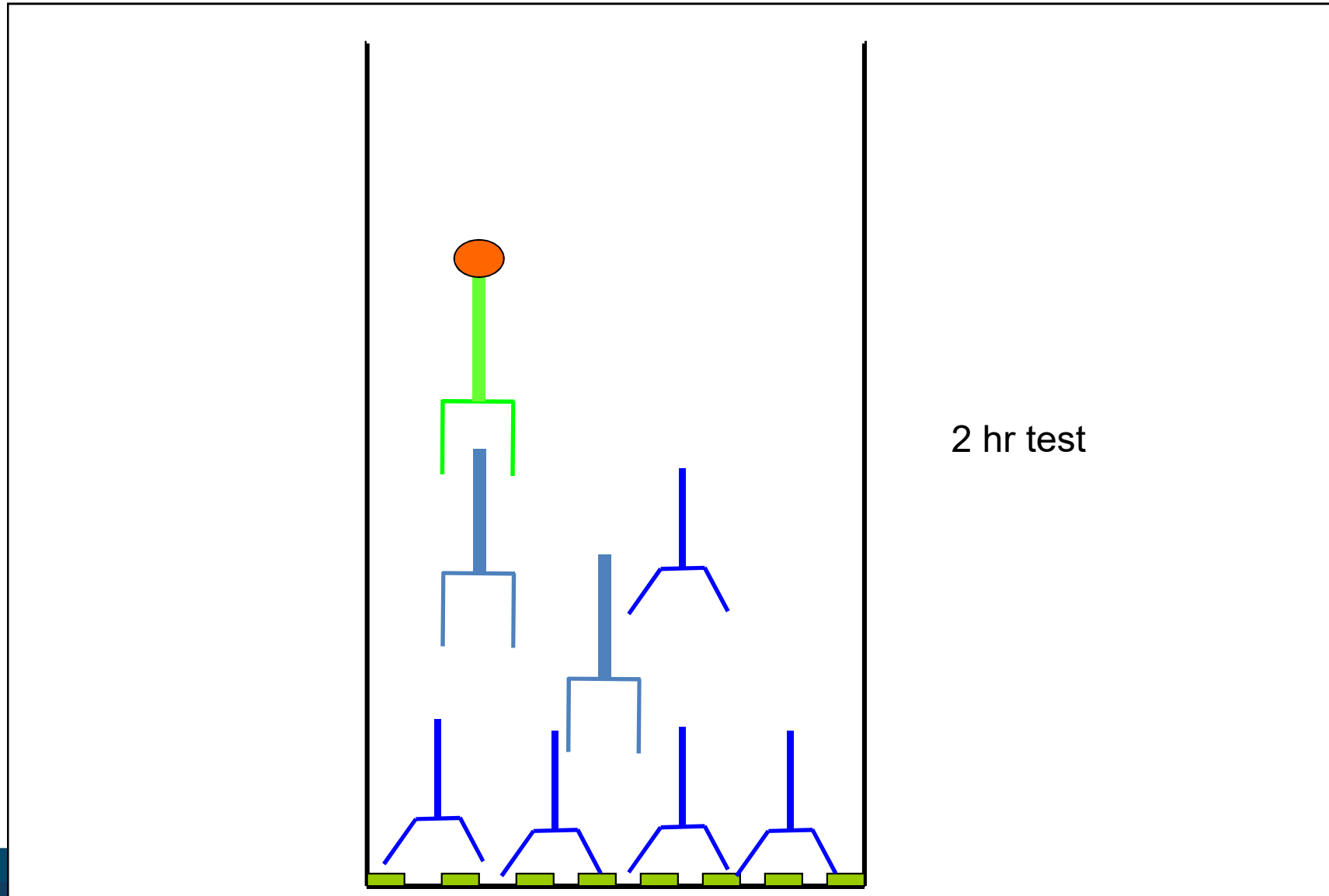


New 3ABC rec protein

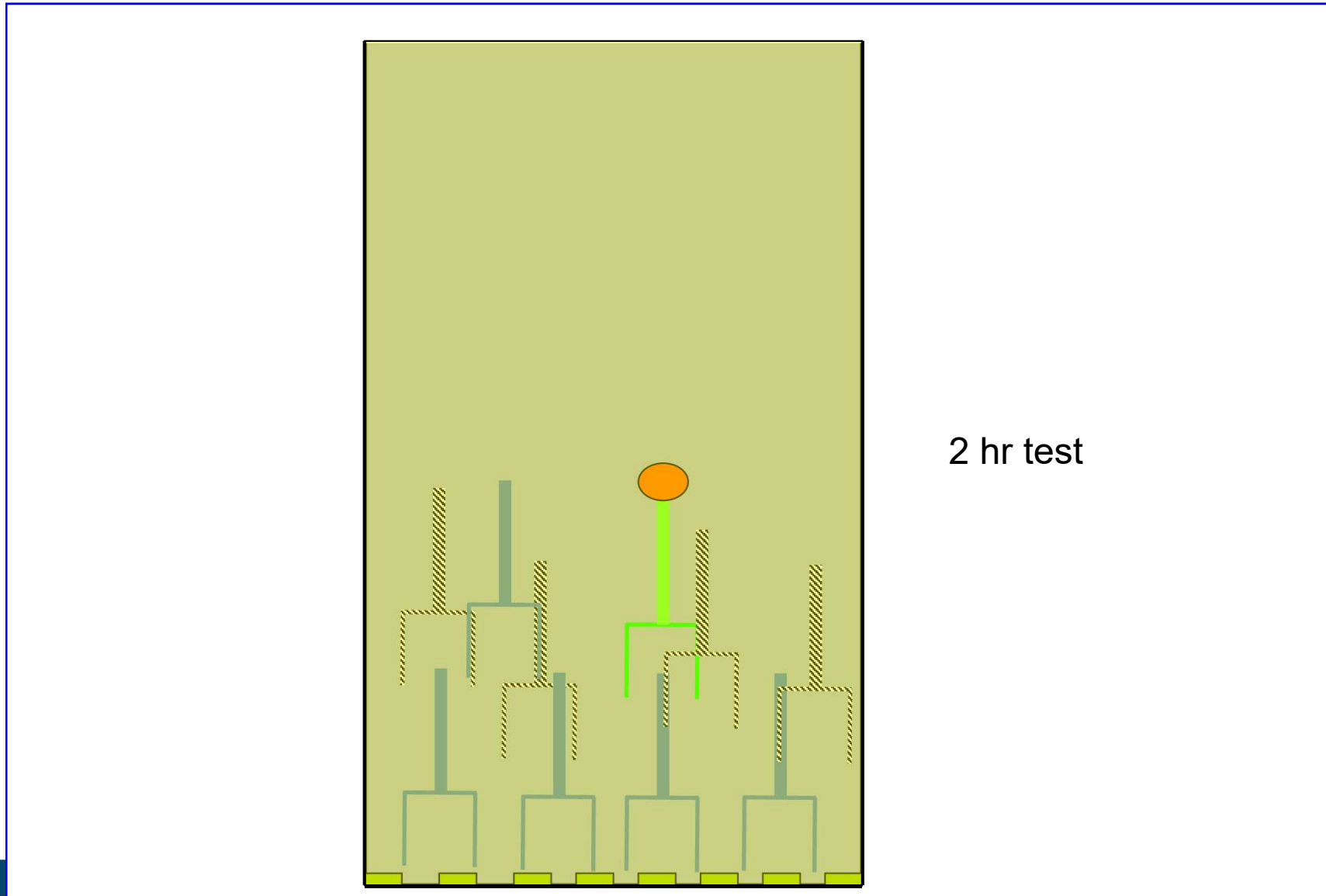




3ABC Solid Phase Blocking ELISA (Positive)



3ABC Solid Phase Blocking ELISA (Negative)





Organización
Panamericana
de la Salud



Organización
Mundial de la Salud

OFICINA REGIONAL PARA LAS Américas

PANAFTOSA

Centro Panamericano de Fiebre Aftosa
Salud Pública Veterinaria



PANAFTOSA / SPV
OPS / OMS



PILOTO EITB19

Caja 2

FA - Prueba Tamiz (3ABC) - Caja 1

Prueba Confirmatoria (EITB) - Caja 2

1 Kit = 440 Pruebas Tamiz e 110 Pruebas Confirmatorias

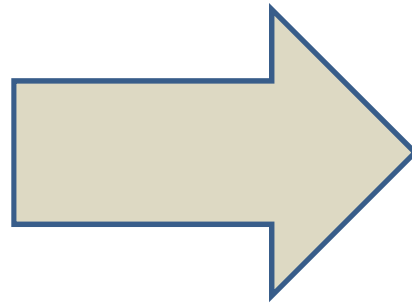
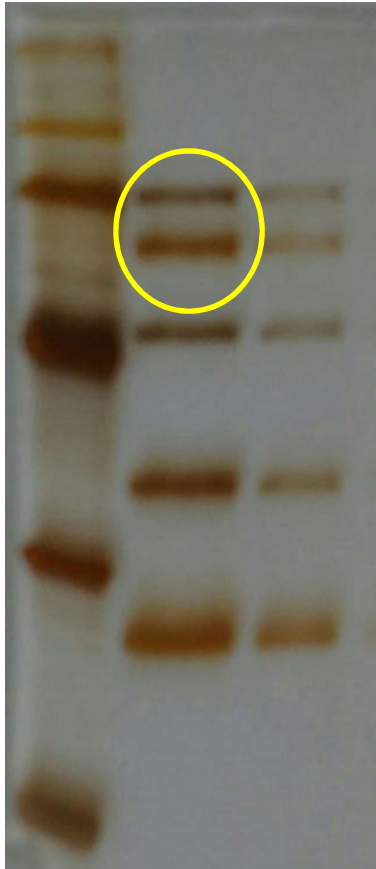
Kit para detección de anticuerpos anti-3ABC, 3D, 2C, 3B y 3A (VFA),
a través de la técnica inmunoenzimática de western blot.

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)
<i>E. coli</i>	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.

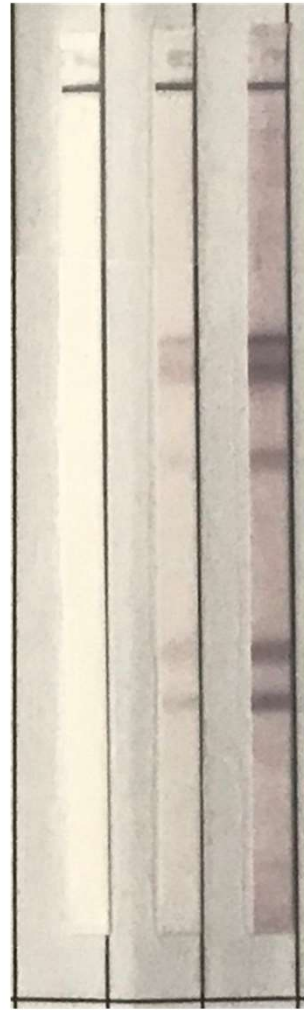
Fab: 03/19
Venc: 03/20
Lote: FAP0119111

EITB vs2.0



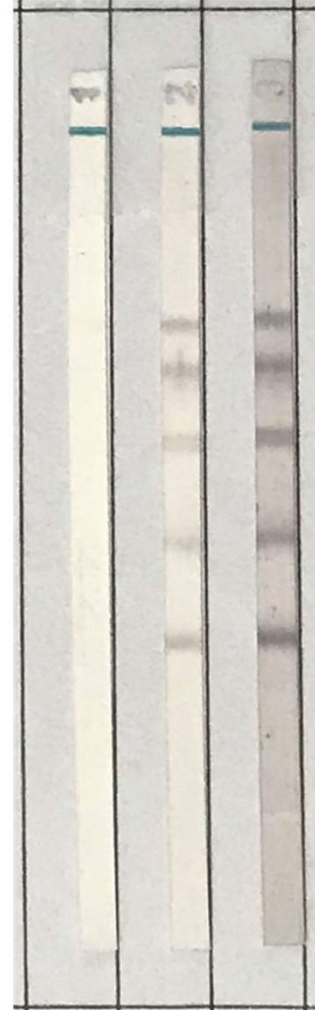
Comparative performance EITB V2.0

CN CP1 CP2



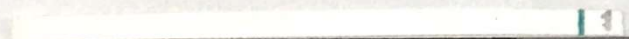






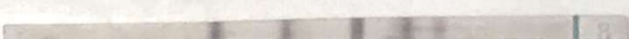


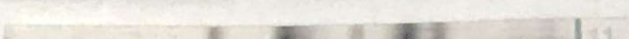
3ABC
3D
2C
3B
3A

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		(+)	(+)	(+)	(+)	(+)
		()	()	()	()	()

Overall results EITB V2.0

Soro	Tiras Testadas	Resultado				
		3A	3B	2C	3D	3ABC
		()	()	()	()	()
CU		✓	✓	✓	✓	✓
CPL		(+)	(+)	(+)	(+)	(+)
CP2		(#)	(#)	(#)	(#)	(#)
		()	()	()	()	()
		()	()	()	()	()
401		✓	✓	✓	(+)	✓
402		✓	✓	✓	(+)	✓
403		✓	✓	✓	✓	✓
404		✓	✓	✓	(+)	✓
405		✓	✓	✓	(+)	✓
406		✓	✓	✓	(+)	✓
407		✓	✓	✓	(+)	✓
408		✓	✓	✓	✓	✓
409		✓	✓	✓	✓	✓

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.		(-)	(-)	(-)	(-)	(-)	

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).

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.



OPAS



OMS

PANAFTOSA



Canadian Food
Inspection Agency

Agence canadienne
d'inspection des aliments

Canada 