Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

New diagnostic tools for FMD in support of PHEFA

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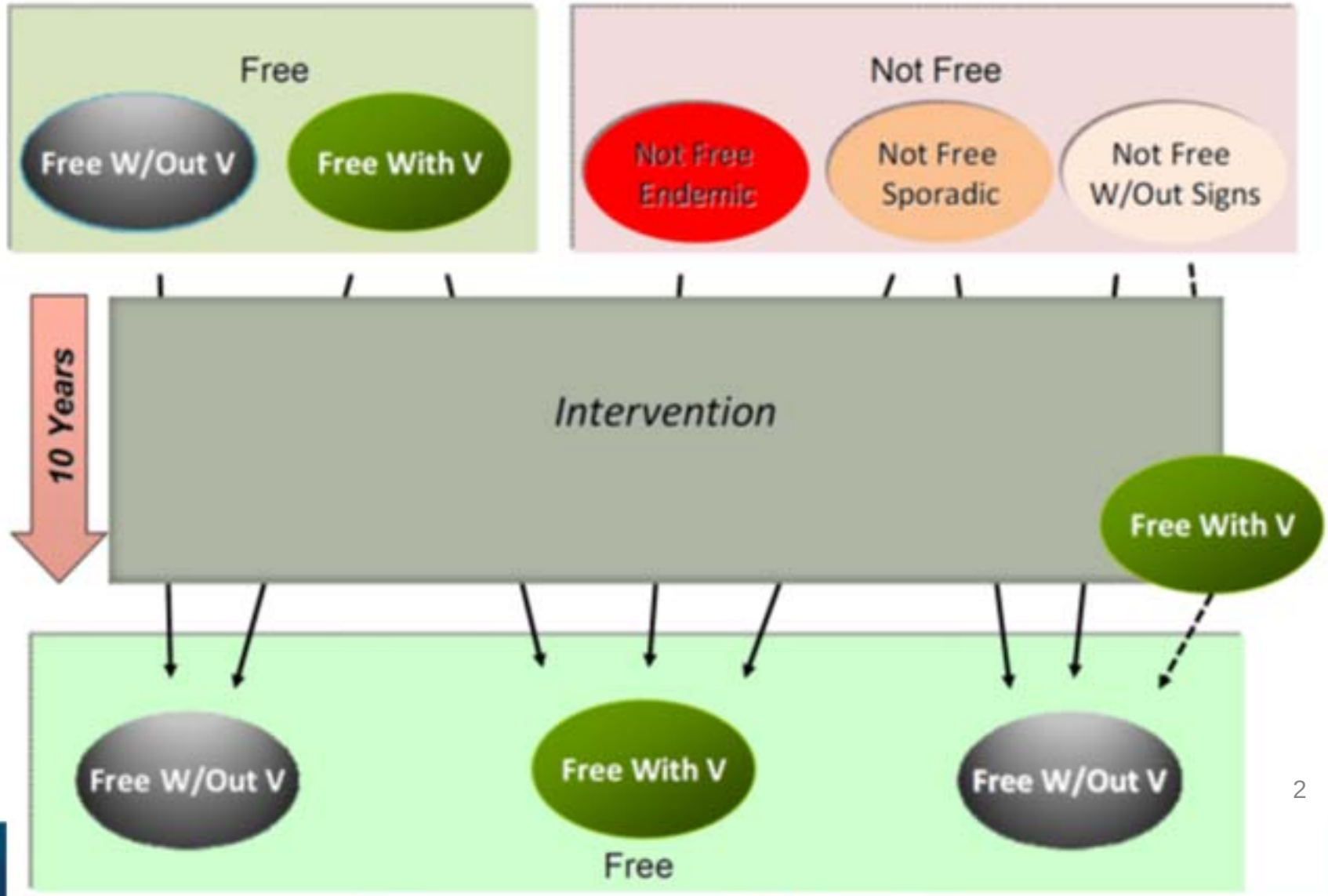
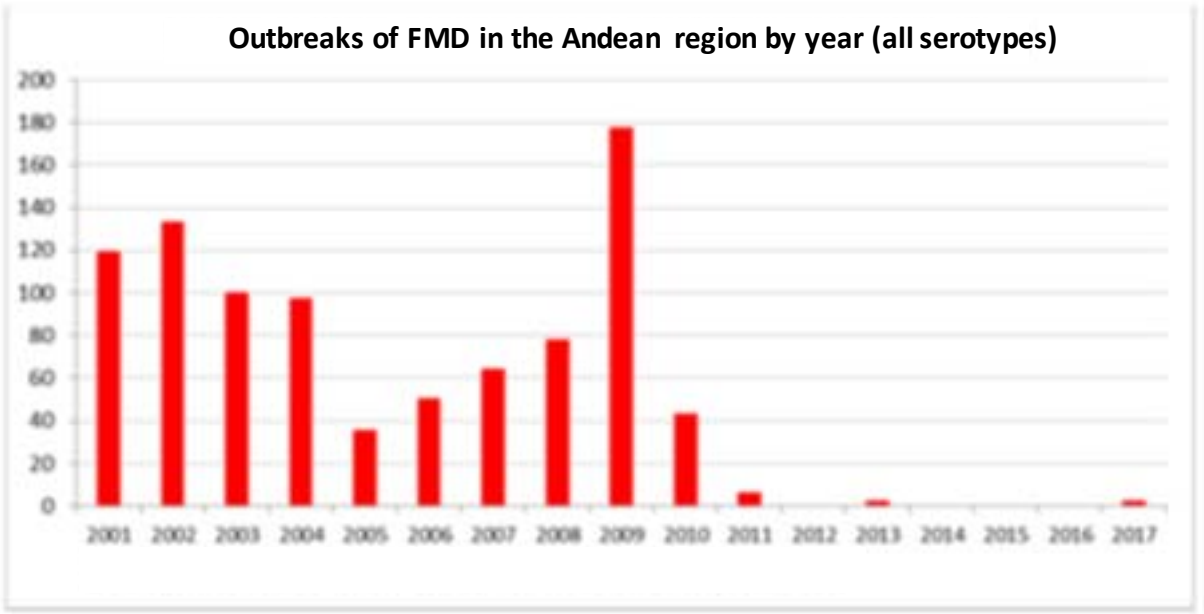
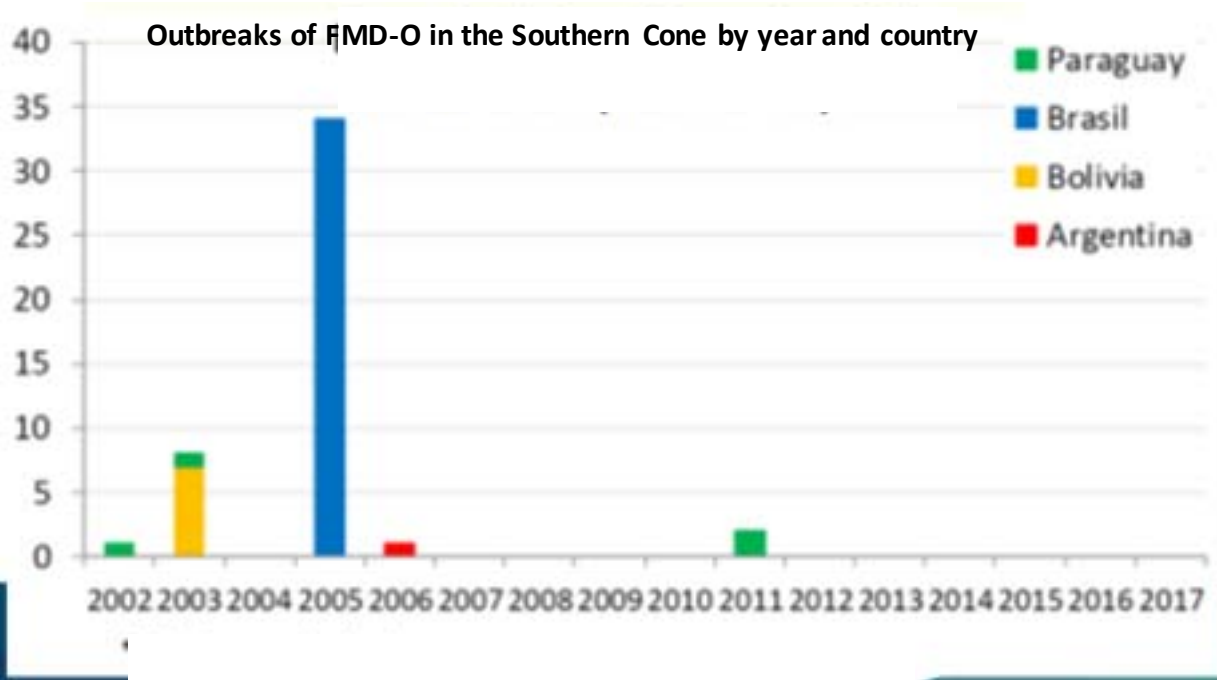
Canada

The Hemispheric Program for the Eradication of Foot-and-Mouth Disease (PHEFA) Action Plan 2011-2020




Outbreaks of FMD in the Andean region by year (all serotypes)
200
180
160
140
120
100
80
60
40
20
0
2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017


Outbreaks of FMD-O in the Southern Cone by year and country
40
35
30
25
20
15
10
5
0
2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017
Paraguay
Brasil
Bolivia
Argentina

PHEFA action plan components proposed to be incorporated into national plans

1. Structure and management of veterinary services
2. Legislation, norms, and regulations
3. Information system
4. Epidemiologic surveillance
5. **Diagnostic laboratories**
6. Immunization and vaccine quality control
7. Sanitary education and public relations
8. Integrated programs in the context of family farming
9. Community participation, with emphasis on the local level

Diagnostic tests in the context of PHEFA

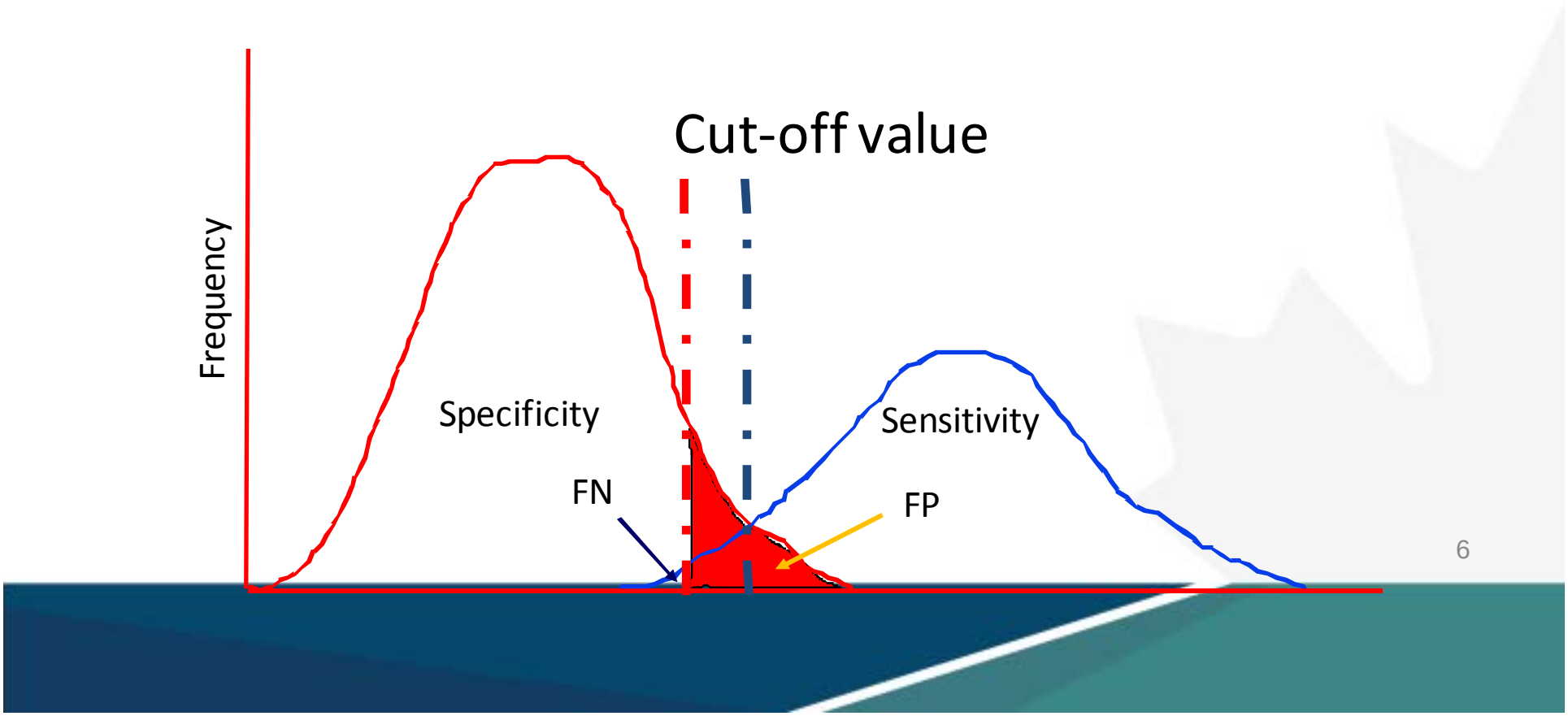
A paradigm shift in the nature of the diagnostic test as the region move to a status of free and free with vaccination.

- Fitness for purpose: High specificity.
  reduce false positives and increase PPV
- Focus in:
  Rapid detection
  Strain characterization

Choosing Between Tests

Use a highly Sensitive test to:

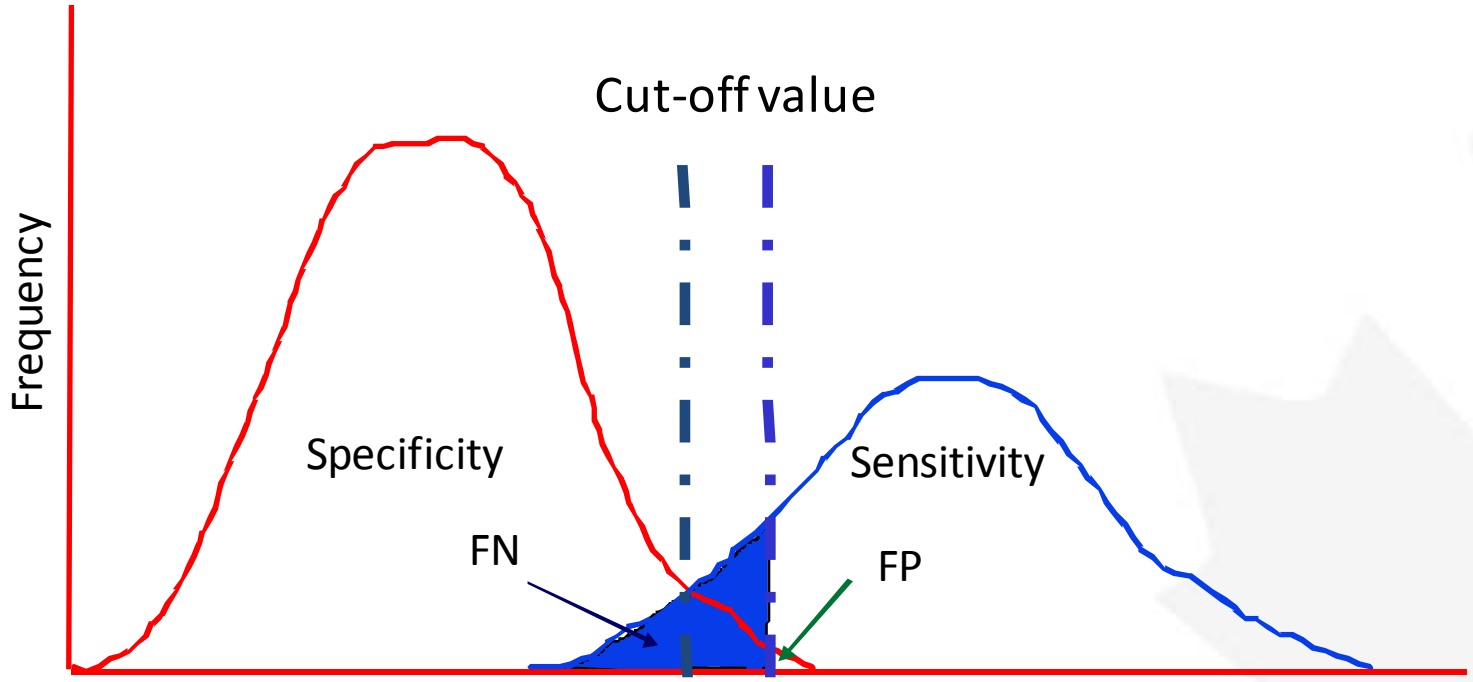
- Have confidence in a negative result
- "rule out" disease (early eradication campaign, screen testing)
- When a FN is dangerous (import/export testing)



Choosing Between Tests

Use a highly Specific test to:

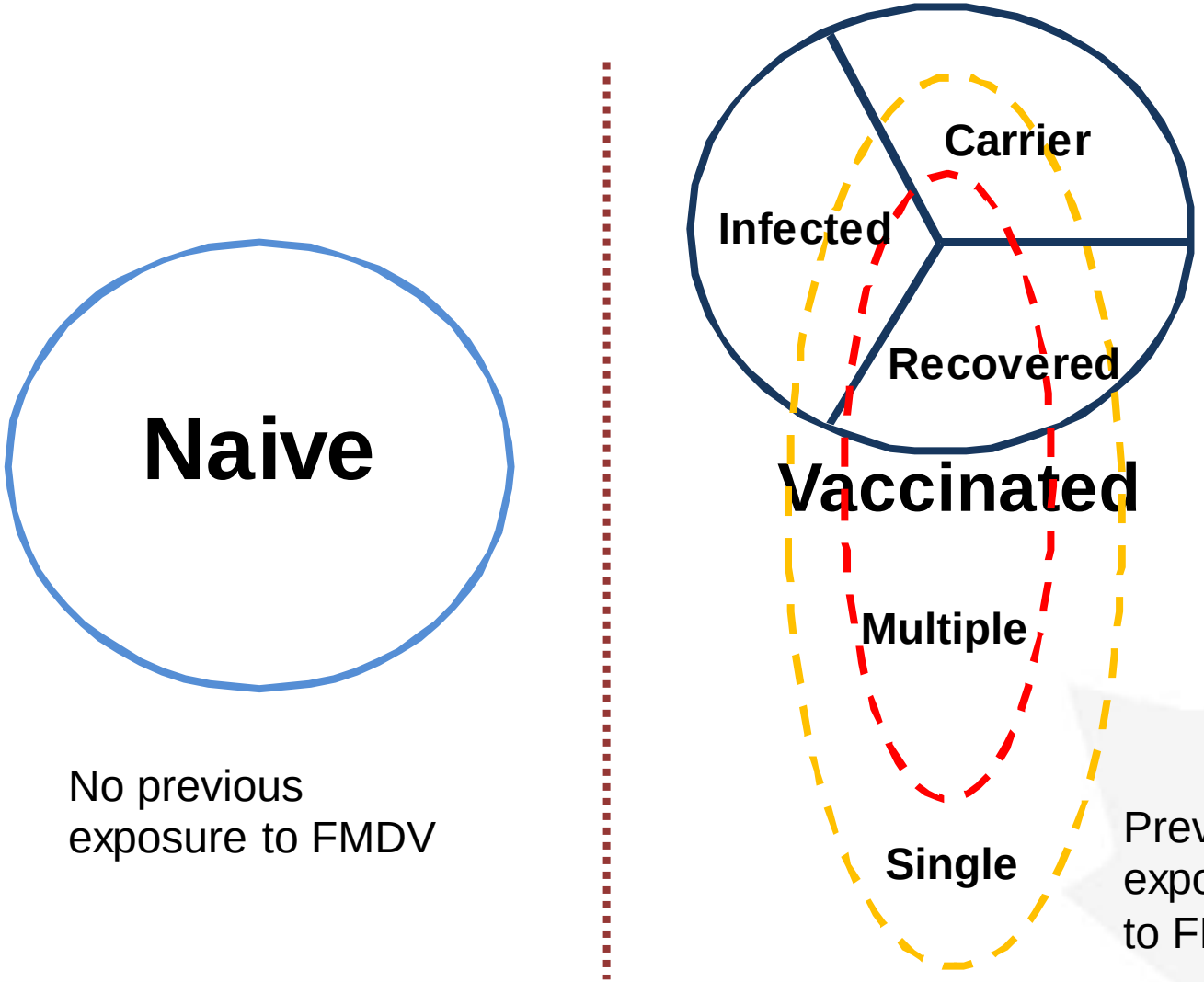
- Have confidence in a positive result
- "Rule In" or confirm a diagnosis (Dx test, late eradication)
- When a FP is dangerous (Disease accreditation, FAD diagnosis)



Serological assays

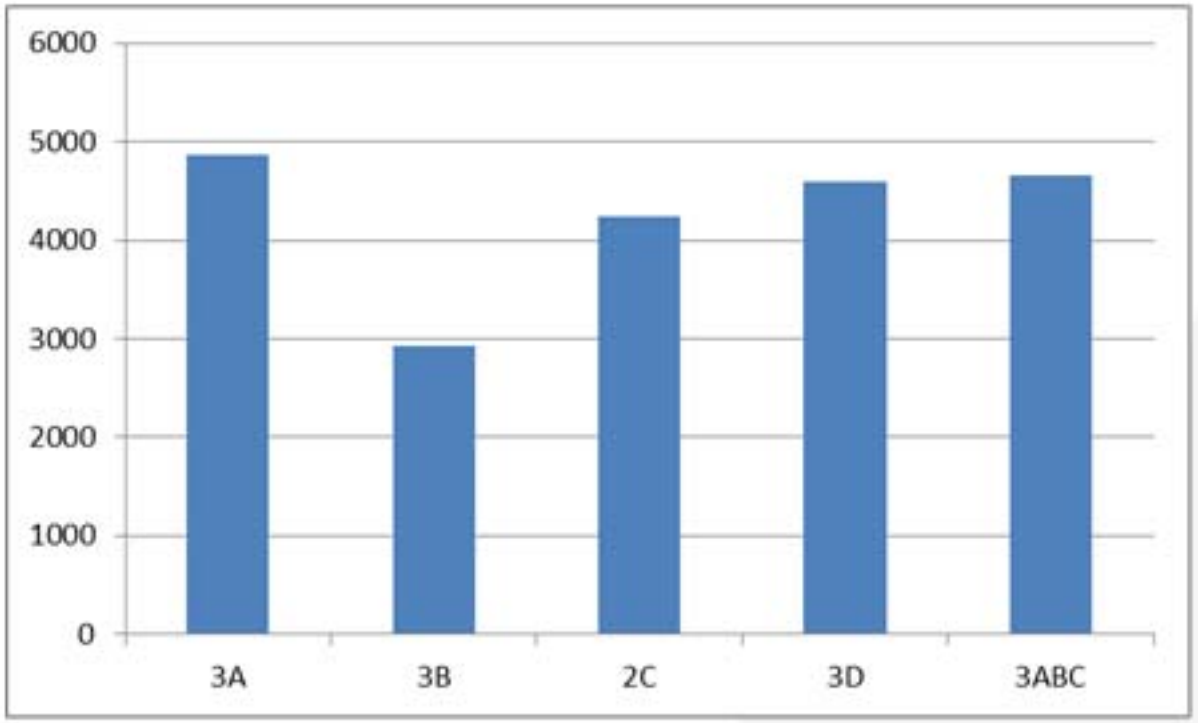
- Targeted towards structural (SP) or non-structural proteins of FMDV
- SP assays – separate assays (VNT or ELISA) required for each serotype
- NSP assays – broadly serotype cross-reactive (ELISA - 3ABC protein and others)

Serological States in FMD



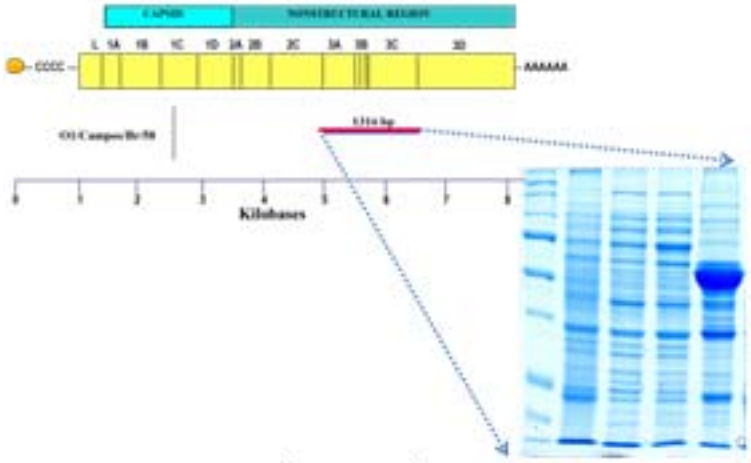
Reactivity to NSPs in EITB (6,184 cattle. PANAFTOSA) Vaccinated population/Post outbreak samples.

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

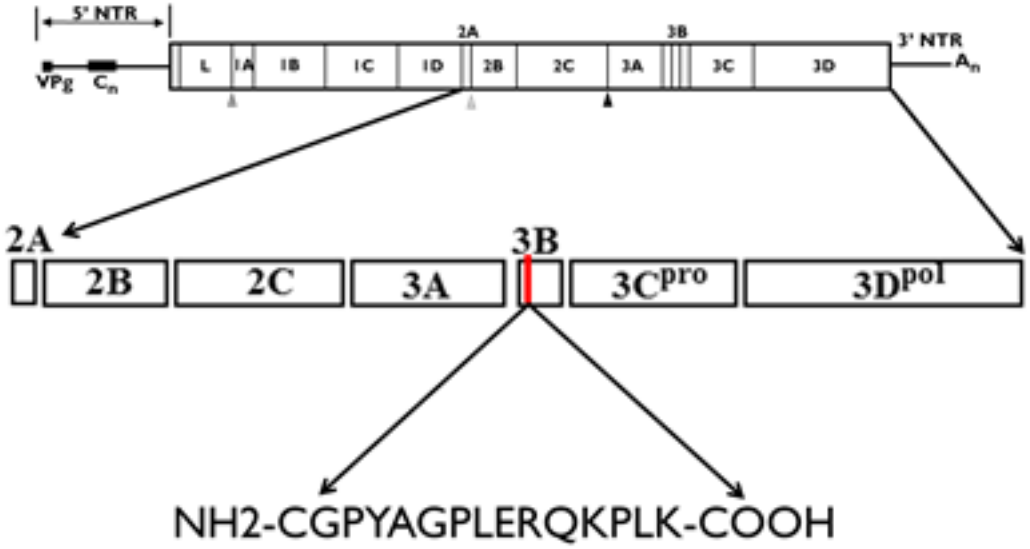


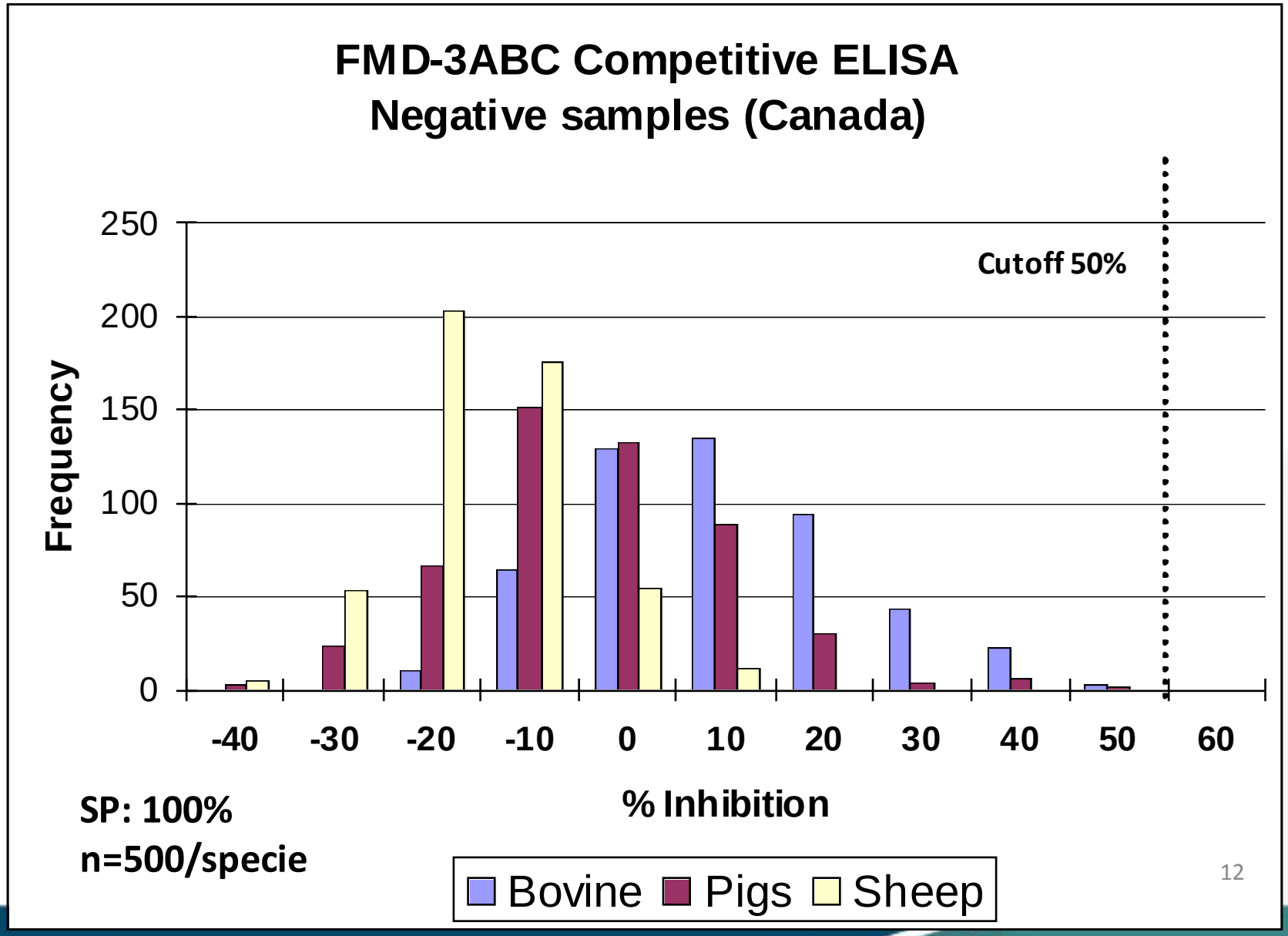
3B competitive ELISA

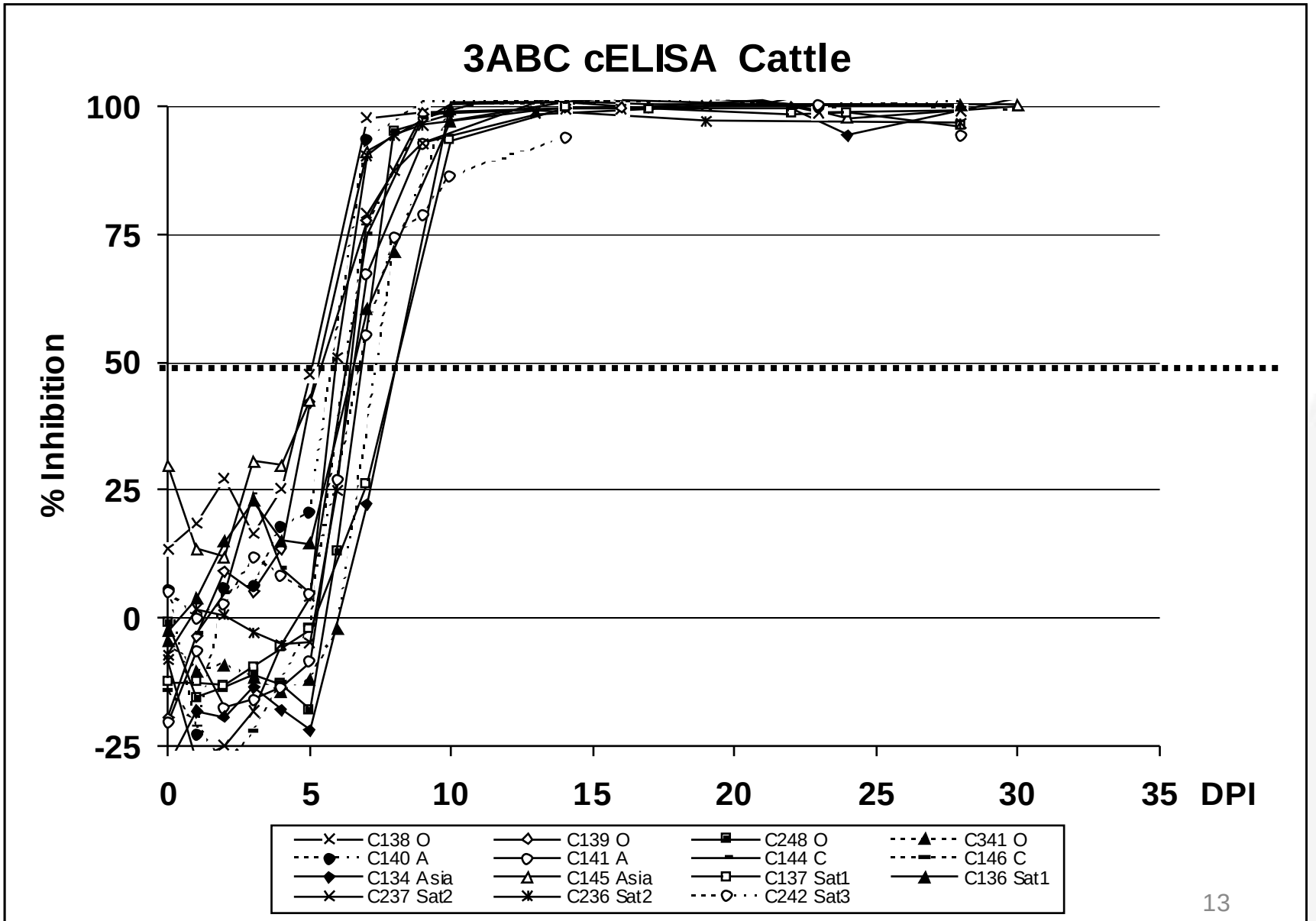
1. Recombinant Protein 3ABC

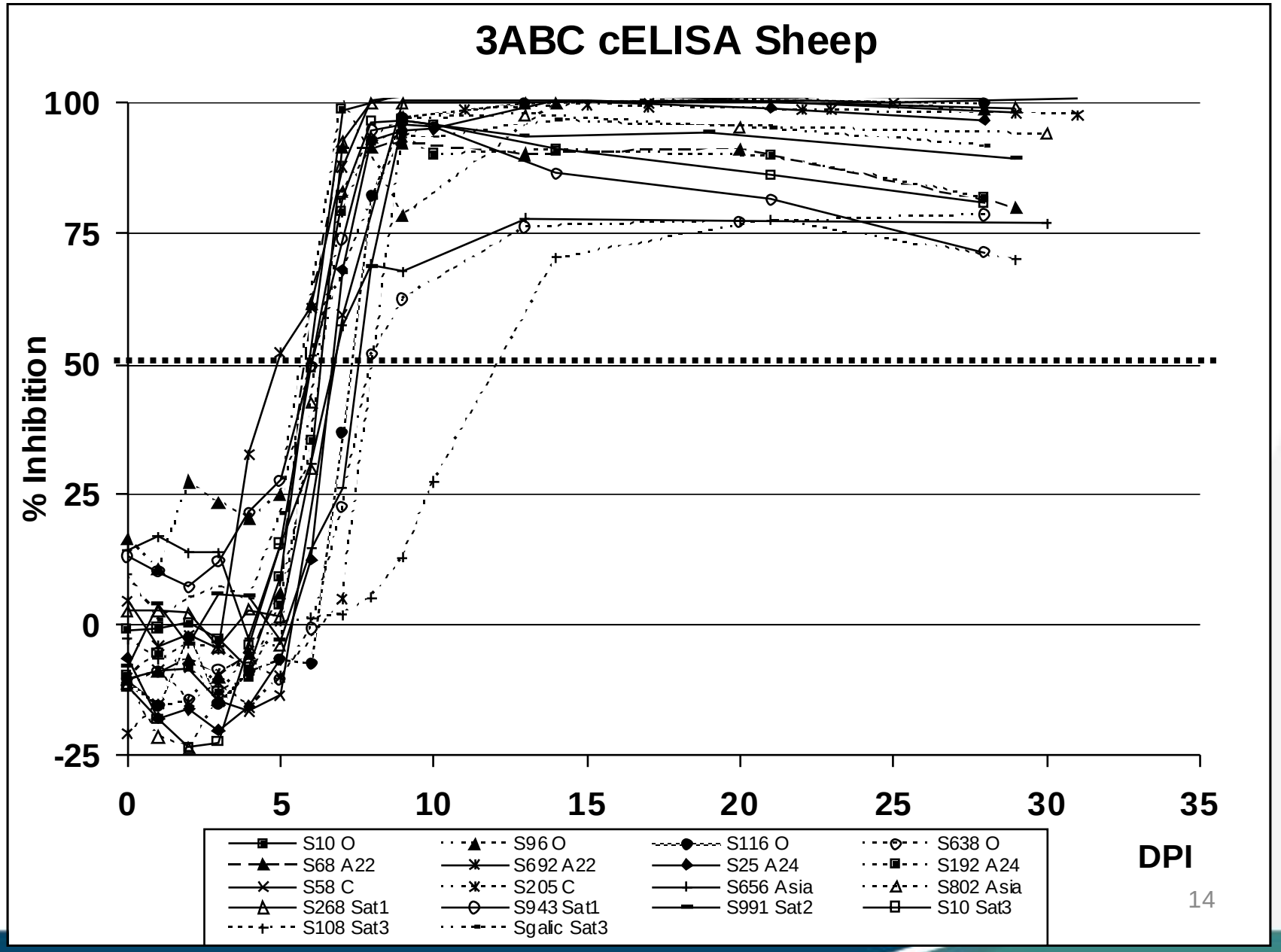


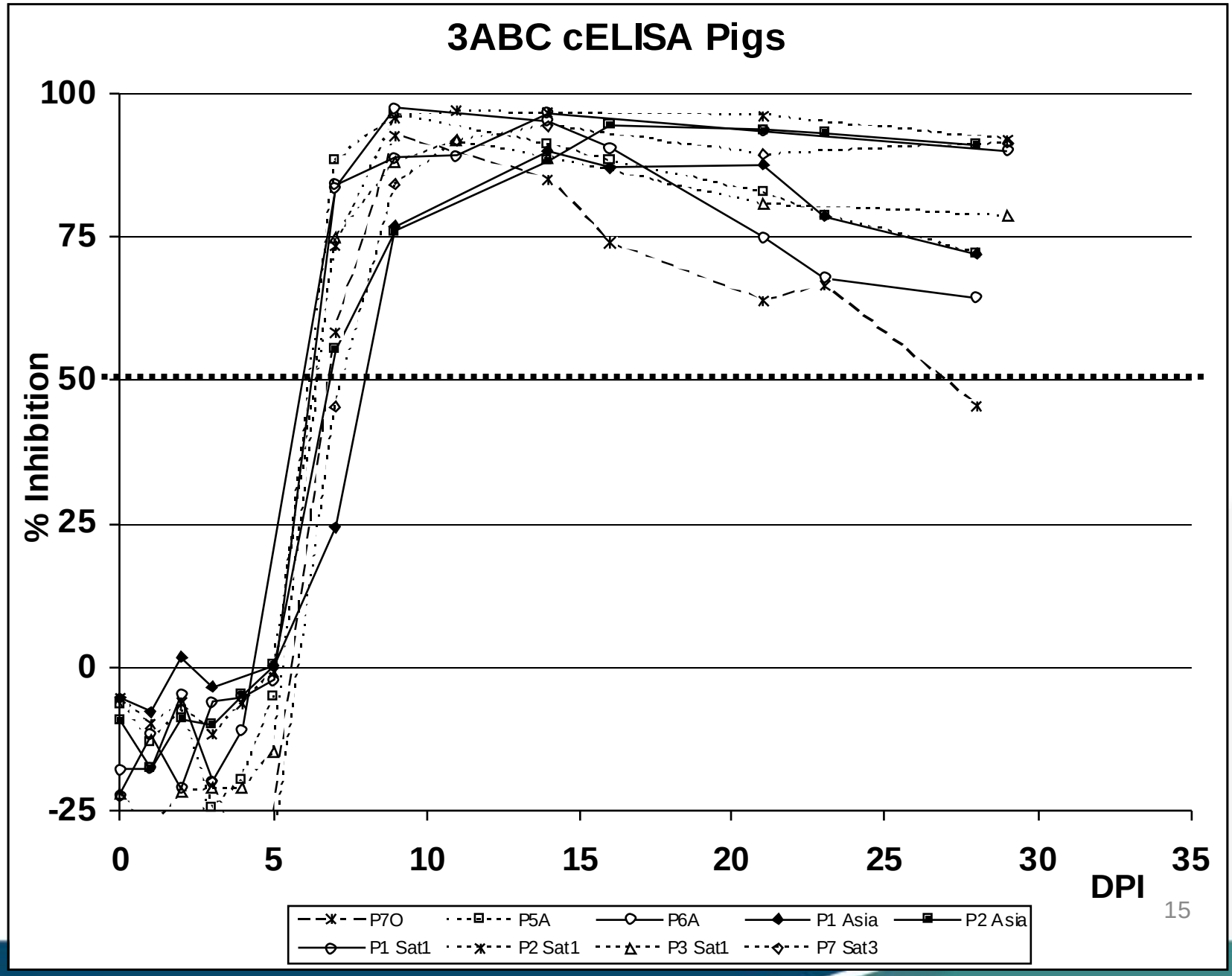
2. FMDV 3B B-cell immunodominant epitope (Mab).

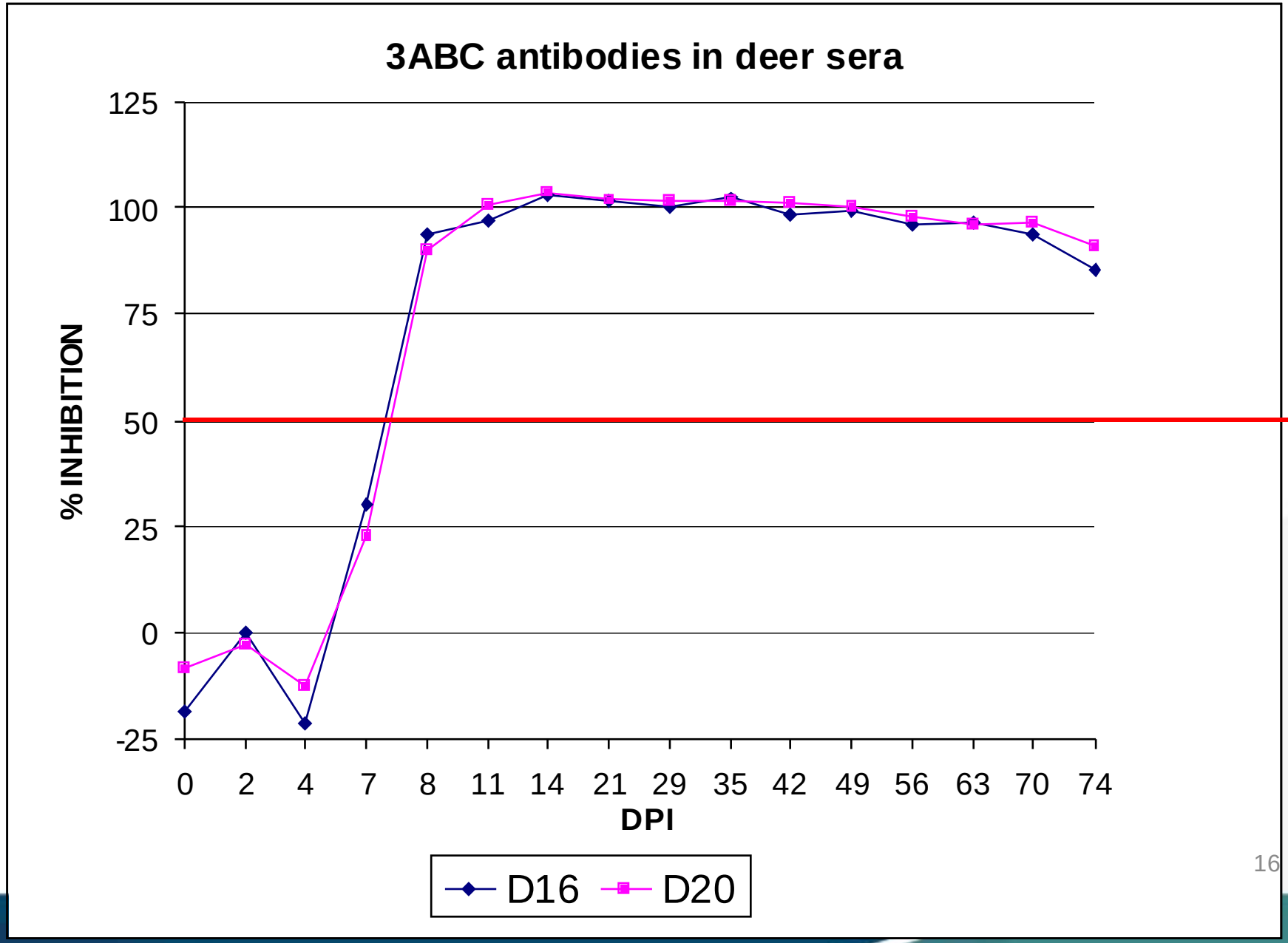



FMD-3ABC Competitive ELISA
Negative samples (Canada)
Cutoff 50%
Frequency
250
200
150
100
50
0
-40
-30
-20
-10
0
10
20
30
40
50
60
% Inhibition
SP: 100%
n=500/specie
Bovine
Pigs
Sheep

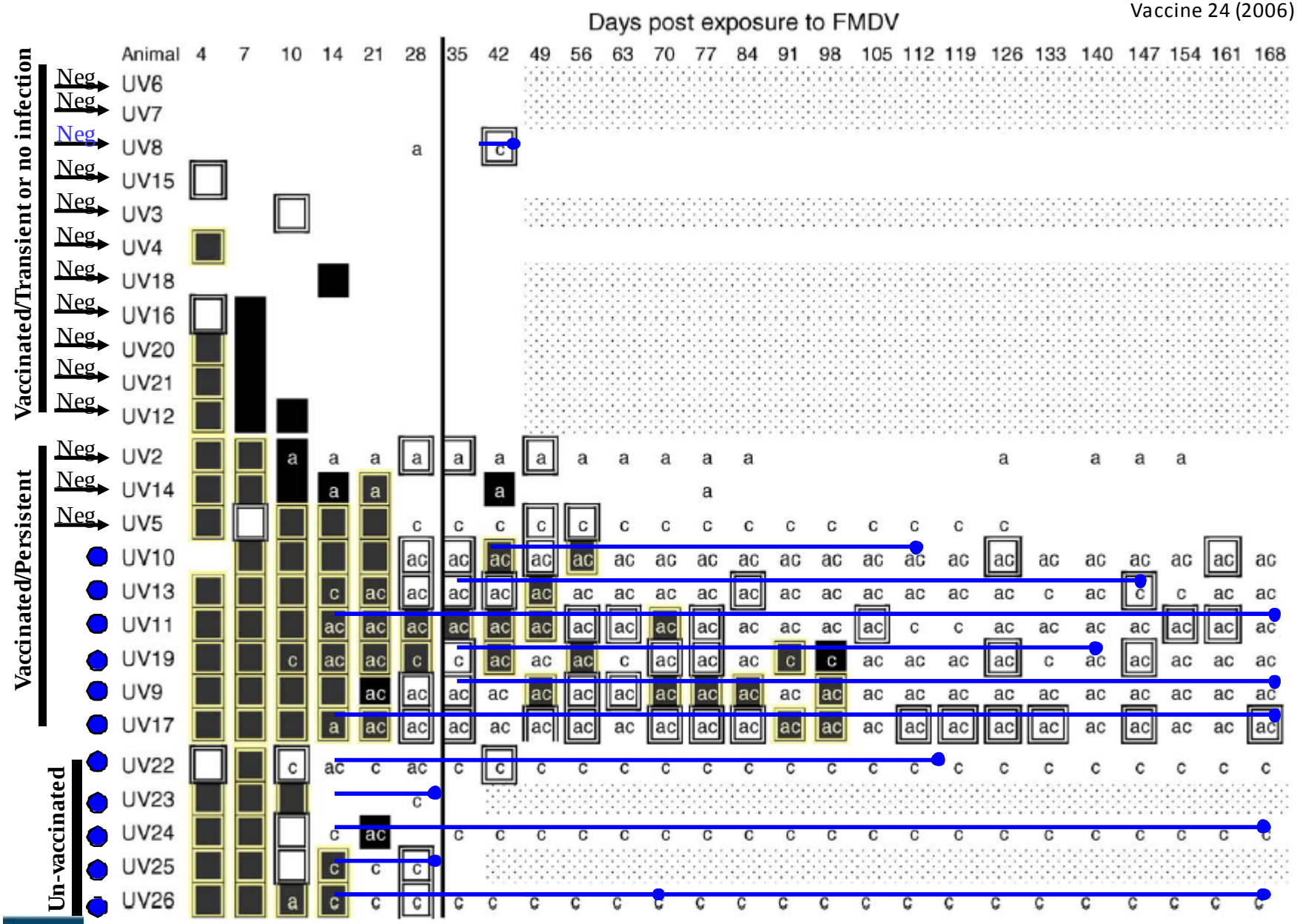

3ABC cELISA Cattle
% Inhibition
100
75
50
25
0
-25
0
5
10
15
20
25
30
35
DPI
C138 O
C139 O
C248 O
C341 O
C140 A
C141 A
C144 C
C146 C
C134 Asia
C145 Asia
C137 Sat1
C136 Sat1
C237 Sat2
C236 Sat2
C242 Sat3


3ABC cELISA Sheep
% Inhibition
100
75
50
25
0
-25
0
5
10
15
20
25
30
35
DPI
S10 O
S96 O
S116 O
S638 O
S68 A22
S692 A22
S25 A24
S192 A24
S58 C
S205 C
S656 Asia
S802 Asia
S268 Sat1
S943 Sat1
S991 Sat2
S10 Sat3
S108 Sat3
Sgalic Sat3

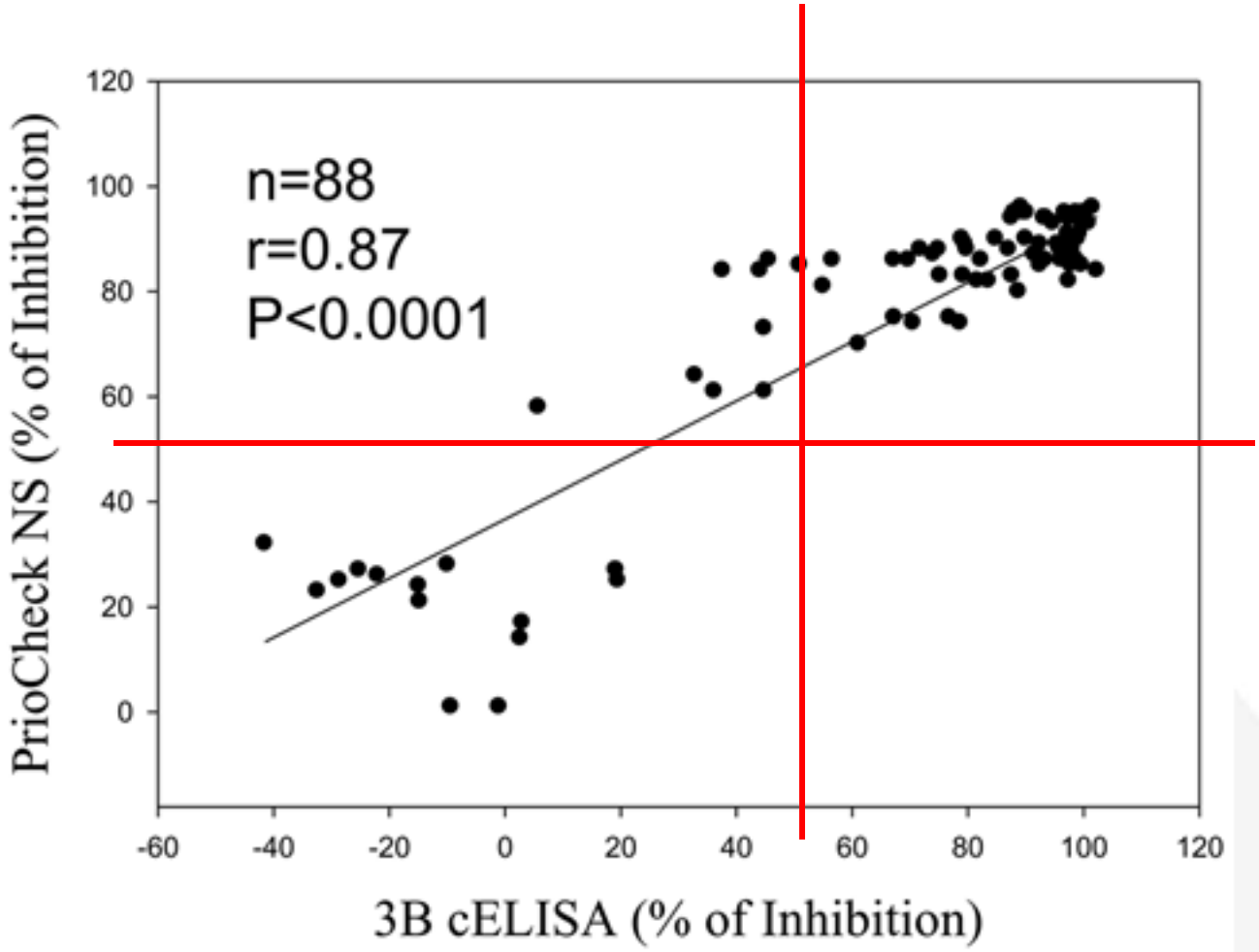

3ABC cELISA Pigs
100
75
50
25
0
-25
% Inhibition
0
5
10
15
20
25
30
35
DPI
P7O
P5A
P6A
P1 Asia
P2 Asia
P1 Sat1
P2 Sat1
P3 Sat1
P7 Sat3


3ABC antibodies in deer sera
% INHIBITION
125
100
75
50
25
0
-25
0
2
4
7
8
11
14
21
29
35
42
49
56
63
70
74
DPI
D16
D20

Vaccine 24 (2006) 1107–1116



(■) VI positive; (a) IgA positive (OD≥0.6); (⬚) Animal dead; (▣) RT-PCR positive; (c) CEDI positive (PI≥50%); (◪) VI +RT-PCR positive.


n=88
r=0.87
P<0.0001
PrioCheck NS (% of Inhibition)
3B cELISA (% of Inhibition)
120
100
80
60
40
20
0
-60
-40
-20
0
20
40
60
80
100
120

Multiple testing

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

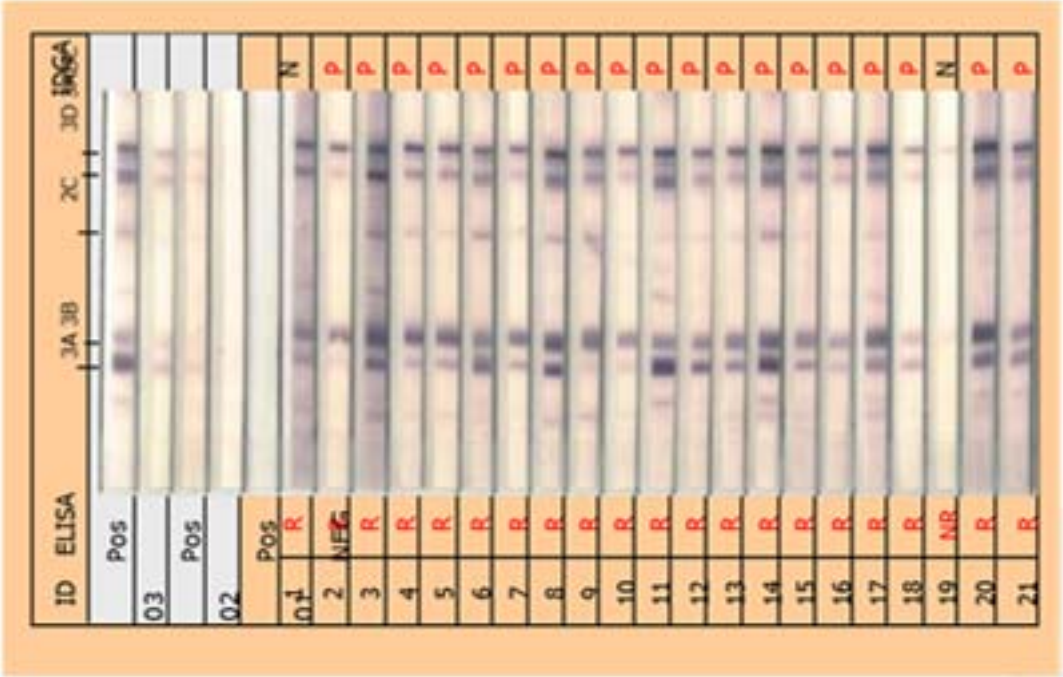
The animal is being asked to "prove" that it is healthy. ⬆Sn and NPV

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

The animal is being asked to "prove" that it has the disease. ⬆Sp and PPV

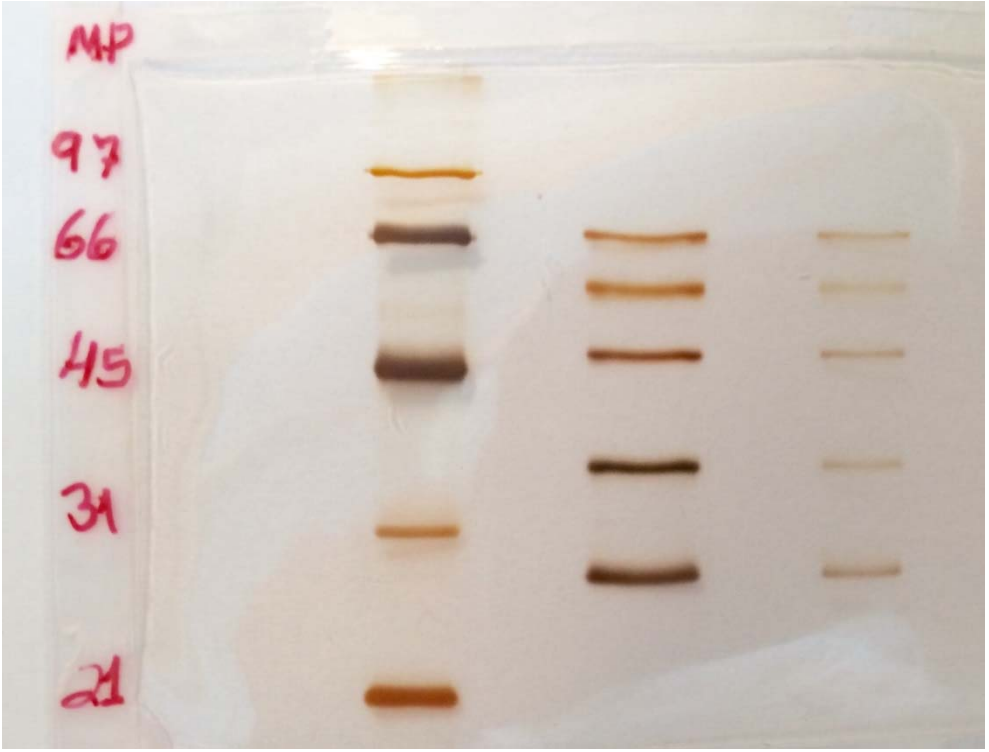
EITB (Enzyme-linked Immuno-electrotransfer Blot Assay)

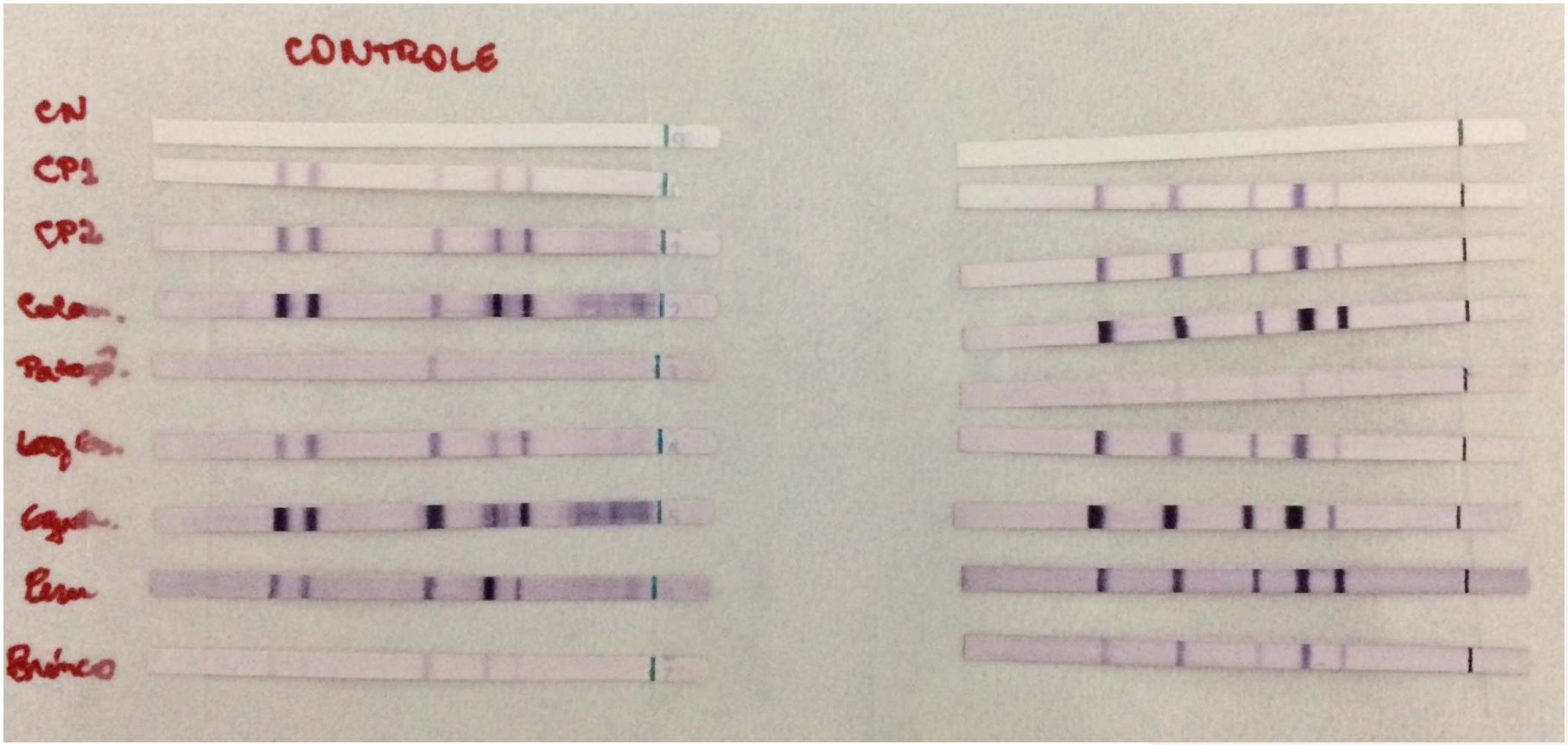
Test with high specificity?????



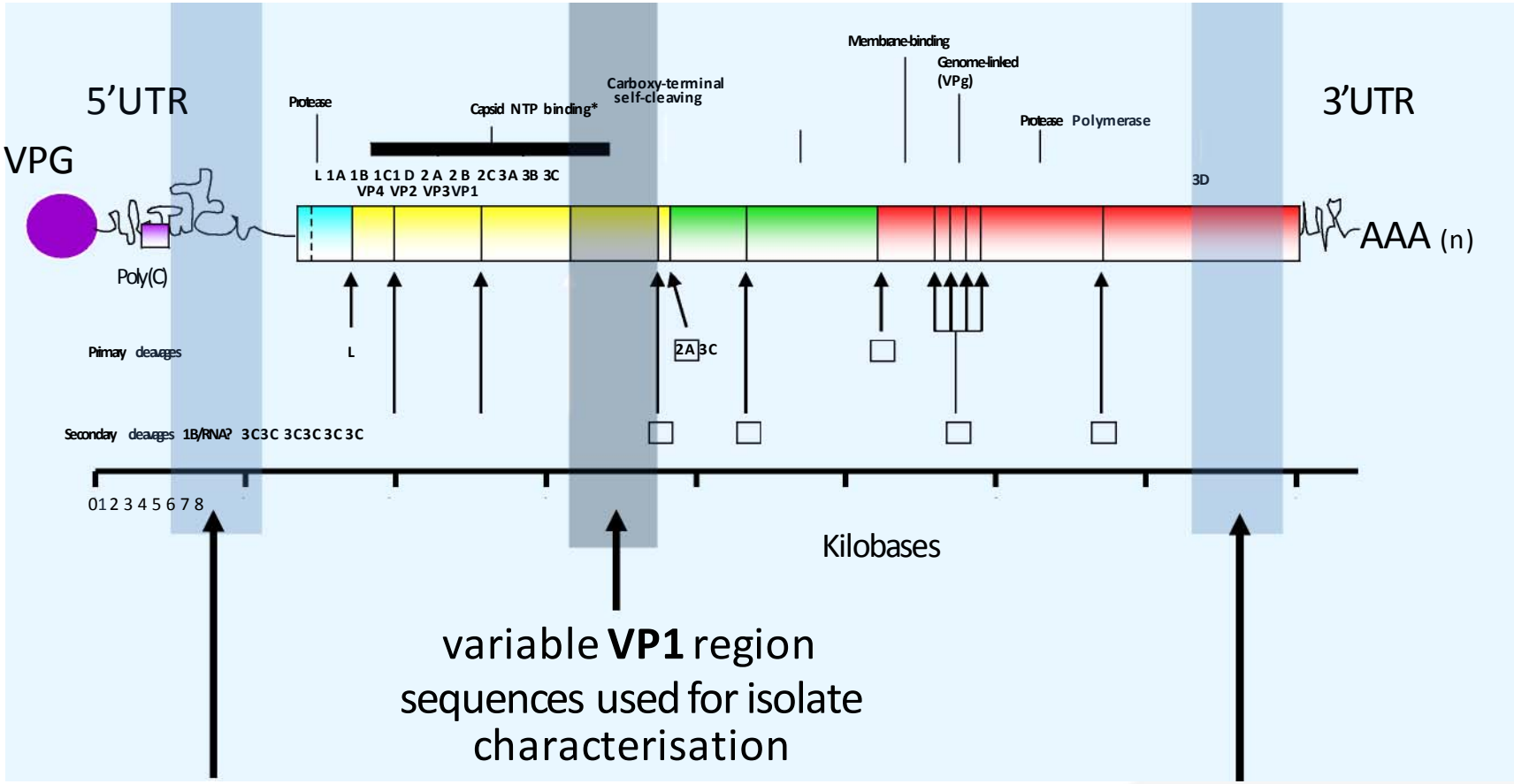
EITB V2.0

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



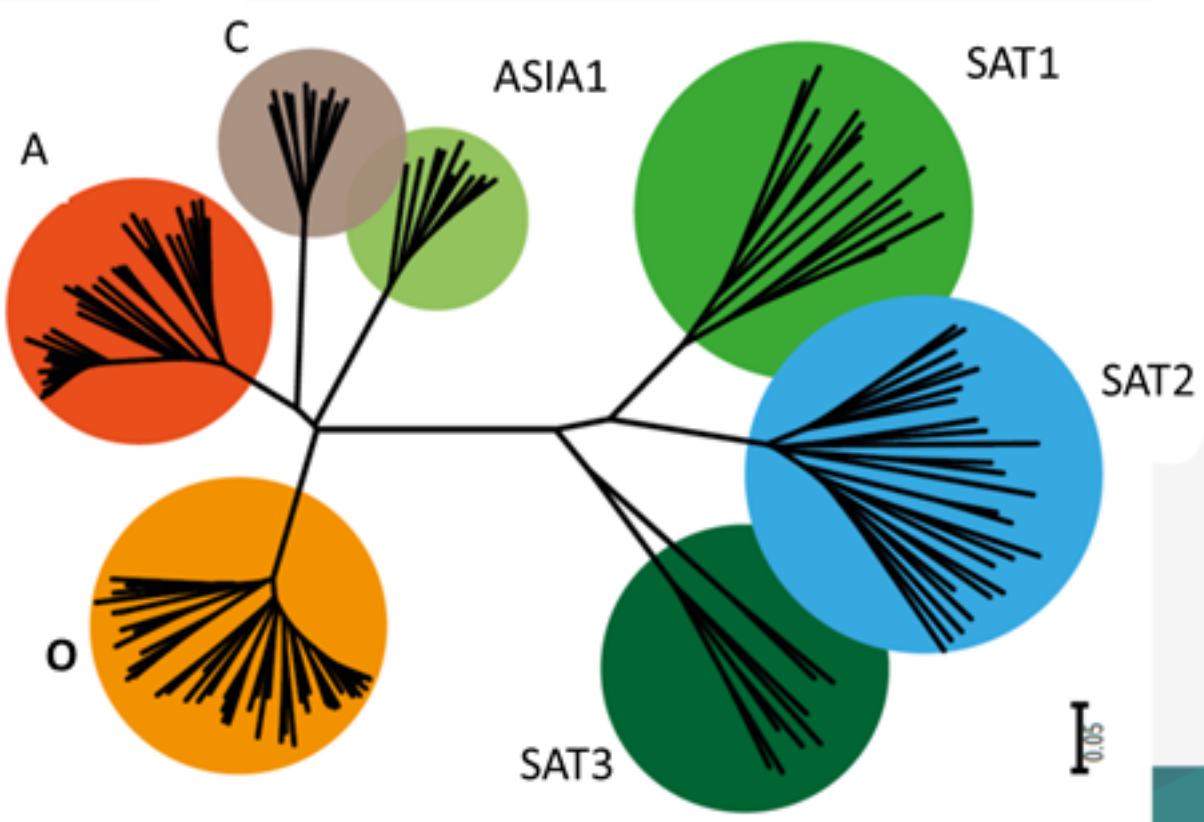

CONTROLE
CN
CP1
CP2
Bruinco

Molecular Diagnostics: Genome detection and sequencing



VP1 sequencing:

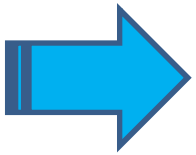
- Enough to classify viruses within serotype and genotype (topotype) based on up to 15% difference in VP1



Full genome sequencing=increased resolution

- Nature of FMDv of rapid spread= rapid evolution
- virus seq. changes 0.5-1% of its genome/year.
  =40-80 nt/year or 1-2 nt/week).

As virus transmit between farms = change is accumulated.

Analysis of the accumulated changes from each infected farm  Identify the transmission pathway

- Obtaining consensus sequence
- Analysis diversity of the virus population at each position along the genome

Field testing vs centralized testing

Suspect case of FMD

LOCAL CLINICAL OBSERVATION

LABORATORY DIAGNOSIS (Regional or NRL)

Suspects secondary outbreaks

Field Tests (LFD)

FMDV antigen detection

FMDV NA detection

Samples for:

- FMDV detection
- Serotype-characterization
- Serology
- In-vitro vaccine matching
- VP1 sequencing
- Full-genome sequencing
- Cross-protection studies

Confirmation of 1st case (initial Dx)

Strain characterization

Active surveillance programmes

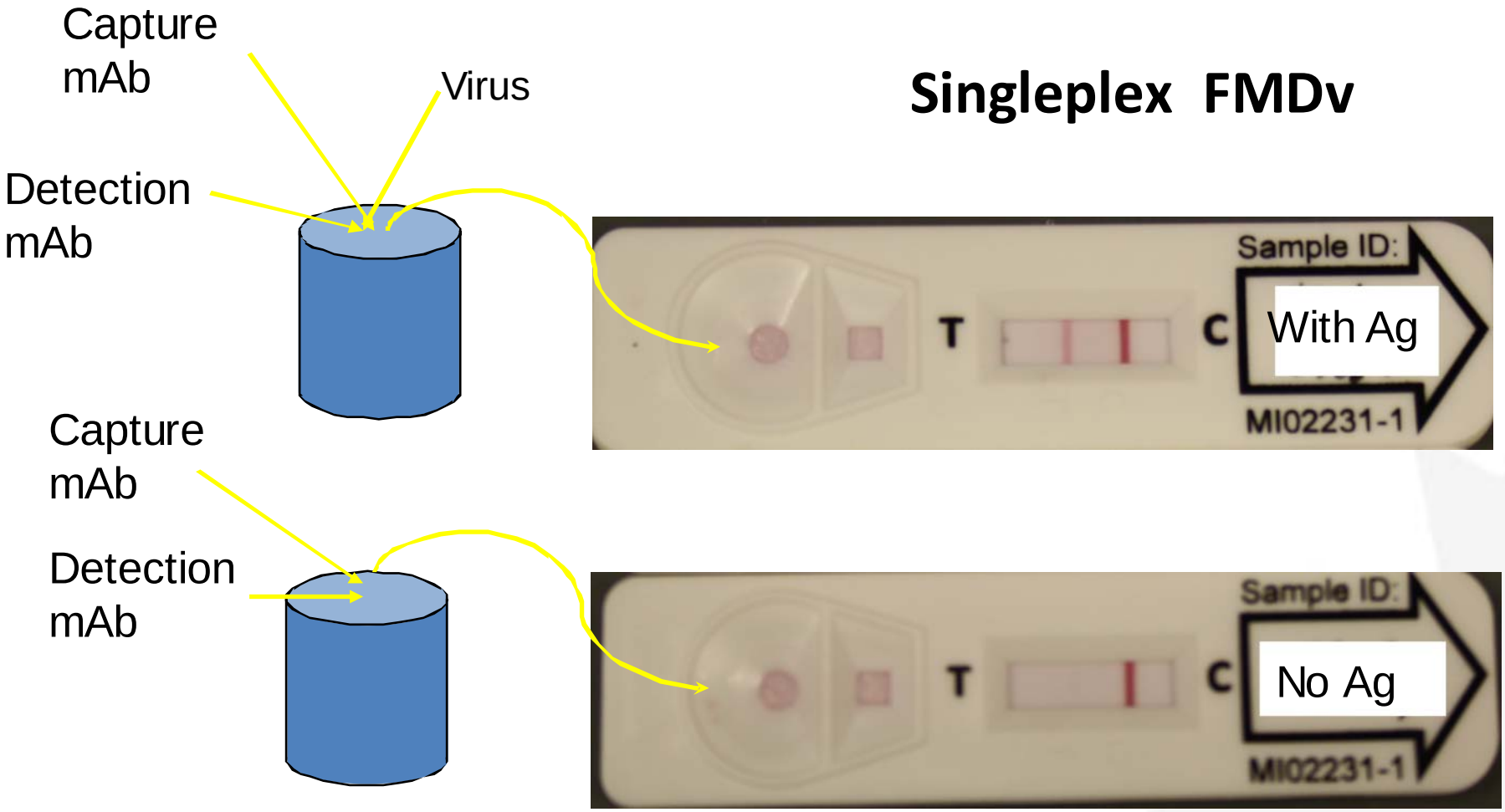
Pen-side testing (PST)
Why do we need field tests for FMD?

- FMD spreads very rapidly
  - –Rapid decision required
  - –Average time to receipt of samples >24hrs
- Shorten time from sampling to results.
- Early Indication of the likely outcome when investigating
- primary cases (confirmation require NRL).
- Improved diagnosis over clinical signs alone.
- Careful result interpretation: Characteristics of the outbreak (clinical disease; epi).
- Lateral Flow Devices
- Portable PCR units

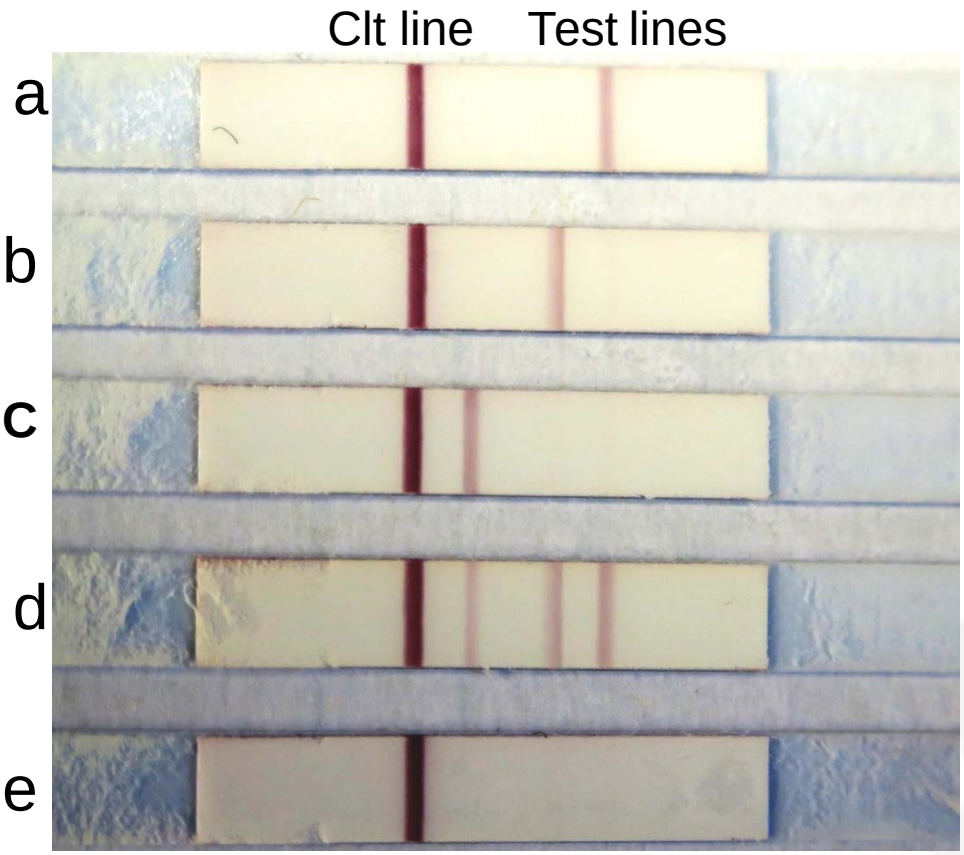
Pen-side testing (PST). Cont..

- How and who will be using Pen–side tests?
- Comparative evaluation and field validation of PST
- Availability of PST, reagents and equipment (including delivery to outbreak).
- Training for use
- Containment risks
- Incorporating PST into the decision-making process
  - Level of proof required to declare new infected/free herds
  - Incorporate additional info (clinical disease. Epi) in the decision.
  - Results reporting

FMD LFD


Capture mAb
Virus
Detection mAb
Capture mAb
Detection mAb
Singleplex FMDv
T
C
Sample ID:
With Ag
MI02231-1
T
C
Sample ID:
No Ag
MI02231-1

Multiplex-LFD strip FMDV serotypes O, A, and Asia 1



Conclusions

FMD Outbreak

Culling
Quarantine
Movement restrictions
Vaccination (with or without subsequent culling)

Regain different levels of FMD-free trading status as soon as possible

Country must demonstrate the effectiveness of the control programs to achieve FMD freedom

FMD-free without vaccination

Demonstrate NO evidence of FMD infection

FMD-free with vaccination

Demonstrate NO evidence of FMD transmission

Absence of viral infection:

Recover status of FMD-free without vaccination (short period of emergency vaccination and then no further use of vaccine).

Identification of carriers becomes critical.

NSP serology Sp and Sn 97-98%. Need to increase to reduce FP rate

NSP Serology in vaccinated+infected cattle: Sn **61%** 15-27dpi/ **23%** 28-100 dpv.

Collection of oesophago-pharyngeal fluids (Probang): Low sensitivity. (VI+PCR)
3 collections 1 week apart (1-3 months post infection)=Sn 80%

NSP testing->confirmatory testing->probang.

Need of more sensitive methodology to detect carrier animals (vacc-infected).

- VNT seroconversion?
- NSP IgM?
- SP IgA
- Cell mediated immune response IFN-ϒ

Vaccine

- Potency evaluation

  Using correlates of protection

- Vaccine strain selection

  Serological methods (mabs?)

  Sequence-based predictions

- Field studies

  Population immunity (vaccination)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