การตรวจวินิจฉัย **ASF** สถานการณ์จริง ณ ปัจจุบัน

น.สพ. ประกิต บุญพรประเสริฐ
กลุ่มไวรัสวิทยา สถาบันสุขภาพสัตว์แห่งชาติ
กรมปศุสัตว์

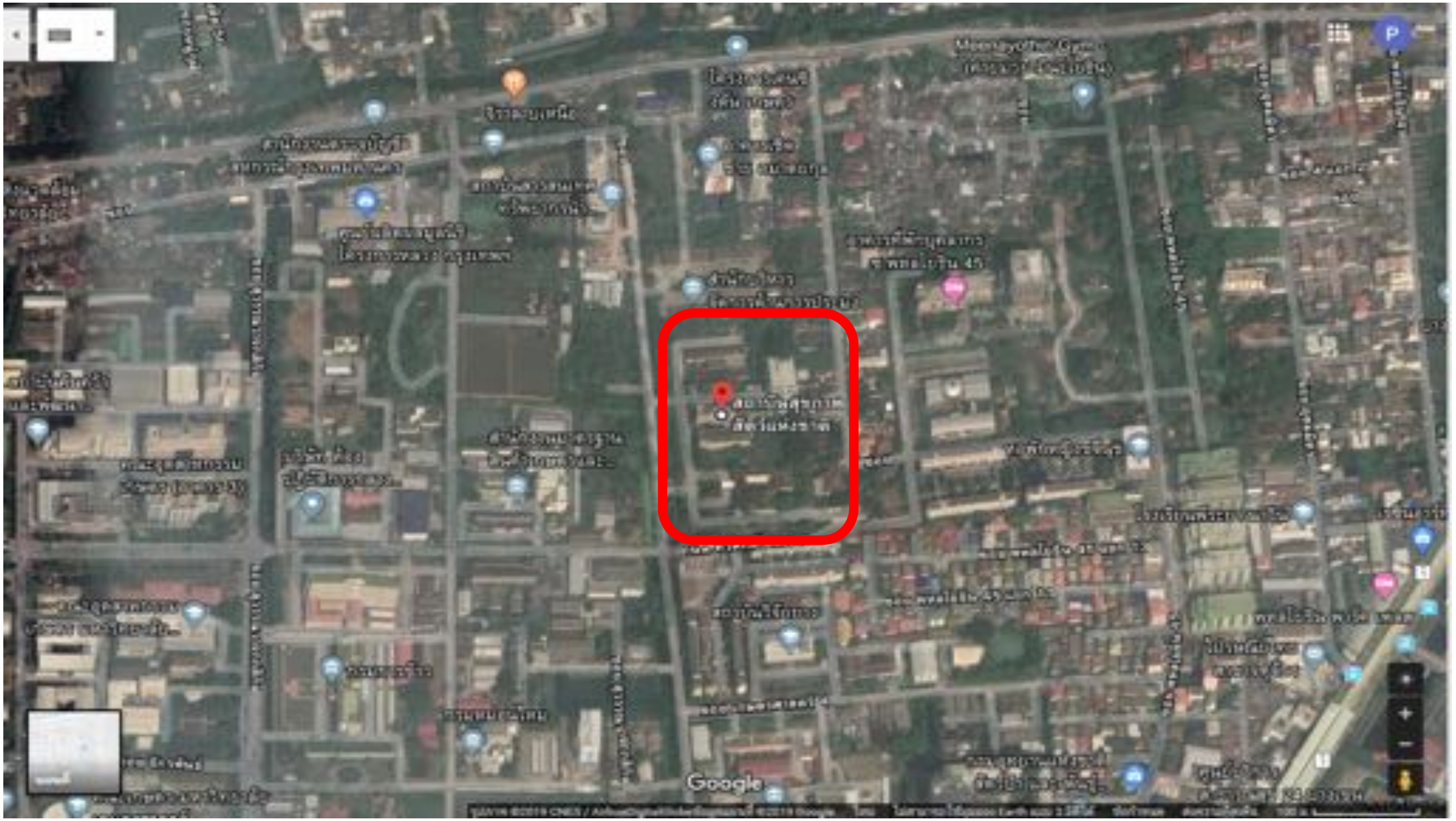
Content

1. Introduction to NIAH.
2. African Swine Fever diagnosis.
3. Detection method of Thailand.
4. Comparison of extraction method.
5. Laboratory network and proficiency testing.
6. Portable qPCR and direct qPCR for ASF.

NIAH

National Institute of Animal Health

Department of Livestock Development, Ministry of Agriculture & Cooperatives, THAILAND

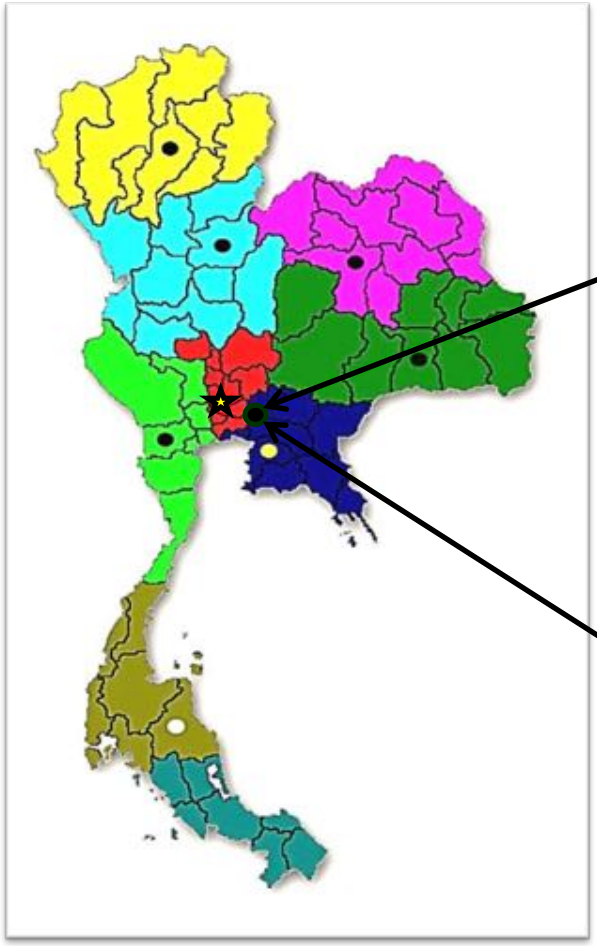

Google

Laboratory Networks

Veterinary Research and Development Center (VRDCs)

- Northern – Upper zone (Lampang)
- Northern – Lower zone (Phitsanulok)
- North East – Upper zone (Khon kaen)
- North East – Lower zone (Surin)
- Eastern – (Chonburi)
- Western – (Ratchaburi)
- Southern – Upper zone (Nakhon si thammarat)
- Southern – Lower zone (Songkhla)

Laboratory Networks



Regional Reference Laboratory for Foot and Mouth Disease in South East Asia (RRL)

- **Pakchong, Nakhon Ratchasrima**

Veterinary Biologics Assay Division (VBAD)

- **Pakchong, Nakhon Ratchasrima**

African Swine Fever diagnosis

AFRICAN SWINE FEVER VIRUS (ASFV)

Complex virus, big size, large genome: 170-190 kb

Complex molecular structure and not well known yet

Genetic variability*

200 nm
More than 100 structural proteins

Macrophages and endothelial are the target cells

NO production of neutralizing antibodies

Lack of effective vaccine yet

24 genotypes*
*The current genotype classification is not related with the virus virulence or the disease evolution (Mur et al. 2016)

ASF diagnosis: key point

- No Vaccine Available → Antibodies = INFECTION
- No Neutralizing Antibodies

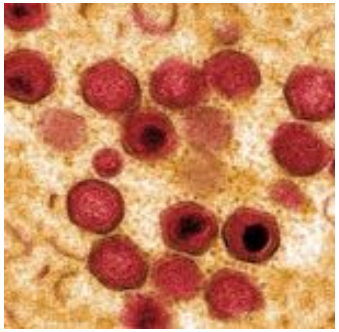
ASFV-specific antibodies do not neutralize virus

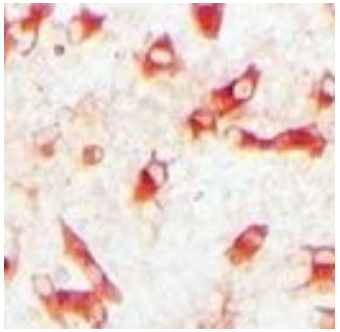
- Viremia for Long Period of time
- Antibodies Persist During Month, even Years ???

Starting in the second week after the infection

Source: Gallardo, 2012

Virus detection technique

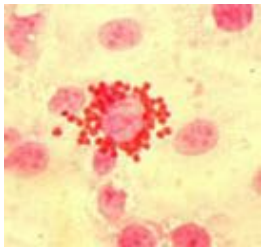




(A) **Detection of virus antigens**

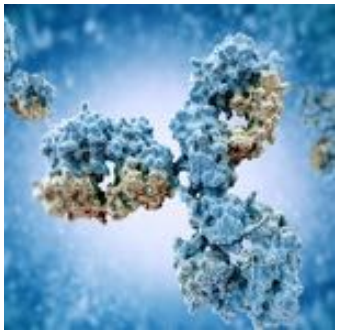
- direct immunofluorescent test
- Antigen ELISA

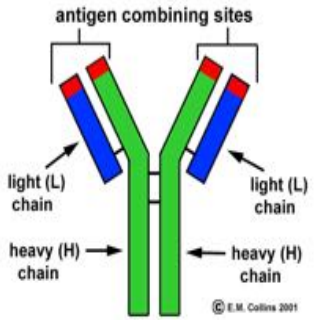
(B) **Virus isolation and Haemadsorption test**



(C) **Detection of virus genome**

Antibody detection technique





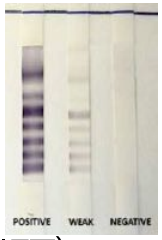
Source: Gallardo, 2012

(A) **Screening by ELISA**

- Indirect ELISA
- Blocking ELISA

(B) **Confirmatory tests**

- Immunoblotting test (IB)
- immunoperoxidase test (IPT)
- indirect immunofluorescent test (IFT)



- The EP402R gene is responsible for the adhesion of swine erythrocytes to infected cell.
- The EP153R is as a stabilizer of this adhesion.

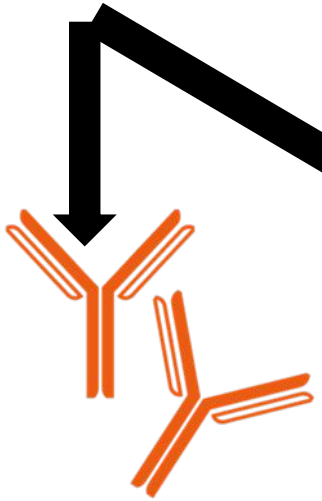
European Union Reference Laboratory for ASF, (EURL-ASF)

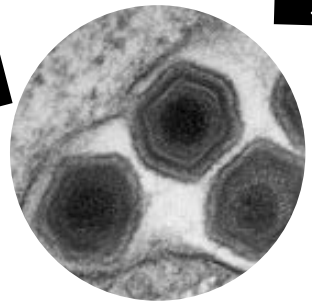
Target samples for ASF diagnosis

1.Serum

2.Blood
(EDTA blood)

3.Organ
(Spleen Kidney Lung Tonsil Lymph node)





4.Tick

Ab detection

Ag detection

Source: Gallardo, 2012

Samples

- Blood and serum

- Lymph nodes

- Spleen

- Lungs

- Kidney

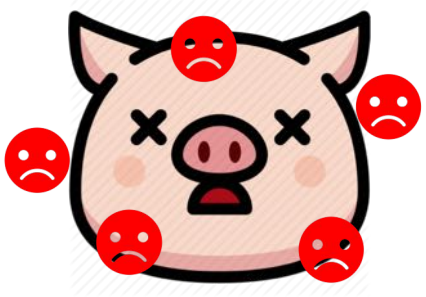
- Oral fluid

- Faeces

ระดับความรุนแรงของโรค ASF จากลักษณะอาการ

- 1. อาการเฉียบพลันรุนแรง (Per acute)
- 2. อาการเฉียบพลัน (Acute)
- 3. อาการกึ่งเฉียบพลัน (Sub acute)
- 4. อาการเรื้อรัง (Chronic)

Peracute



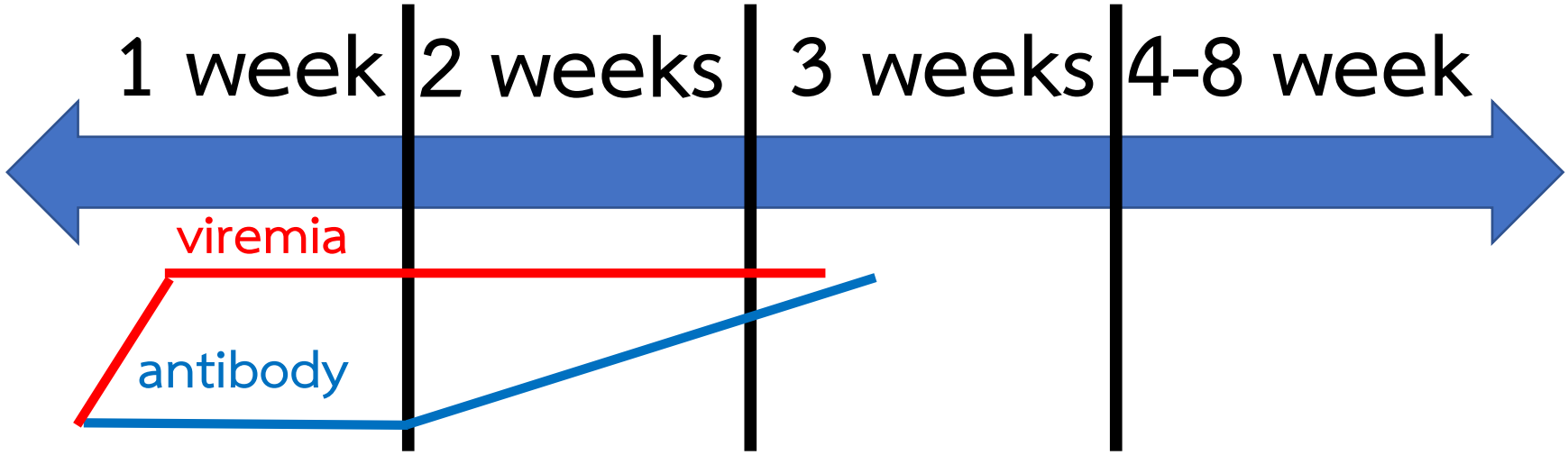
☑ ตายภายใน 24-48 ชั่วโมง

☑ ติดเชื้อสายพันธุ์รุนแรงสูง

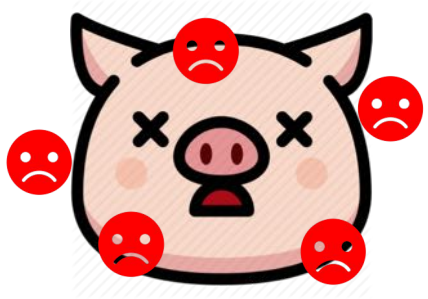
☐ PCR/FAT

☐ VI

Source: Gallardo, 2012



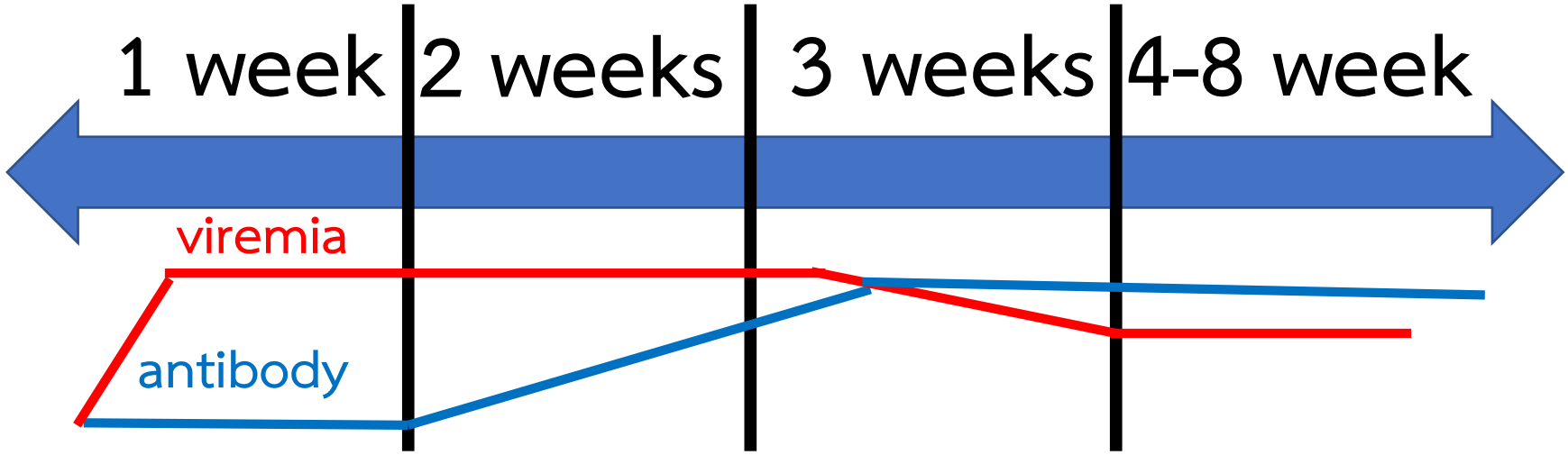
Acute



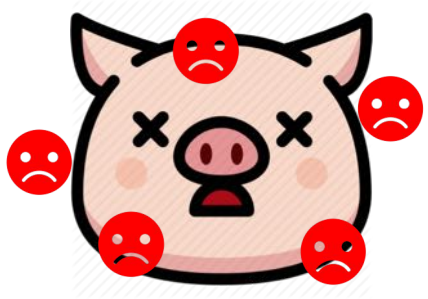
- ☐ PCR/FAT
- ☐ VI
- ☐ ELISA
- ☐ IB/IPT/IFA

- ☑ ตายภายใน 6-13 วัน จนถึง 20 วัน
- ☑ ติดเชื้อสายพันธุ์รุนแรงสูง

Source: Gallardo, 2012



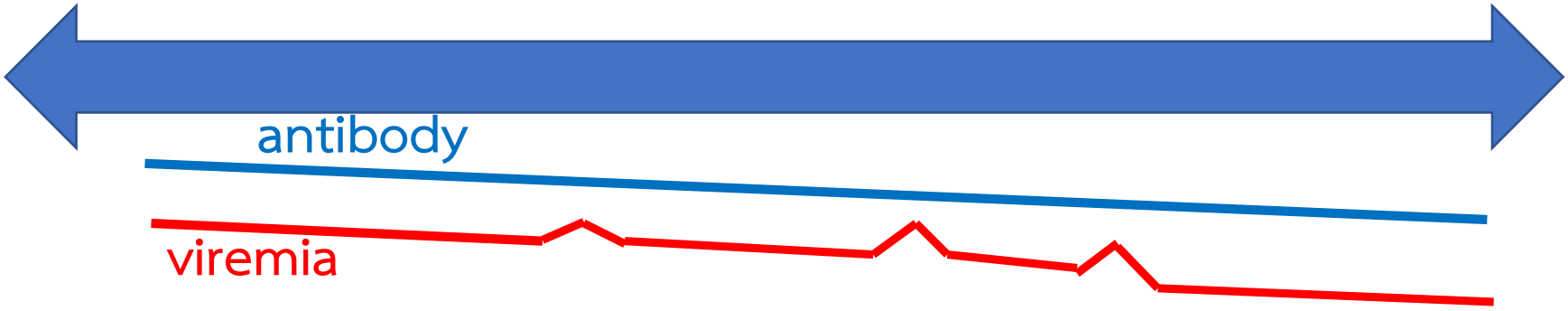
Subacute



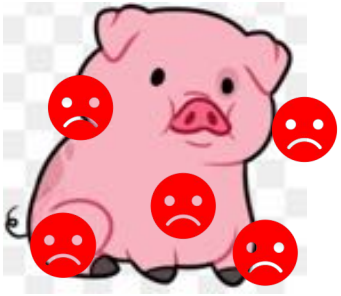
- ☑ ตายภายใน 15-45 วัน
- ☑ ติดเชื้อสายพันธุ์รุนแรงปานกลาง
- ☑ อัตราการตาย 30-70%

- ☐ PCR/FAT
- ☐ VI
- ☐ ELISA
- ☐ IB/IPT/IFA

Source: Gallardo, 2012



Chronic

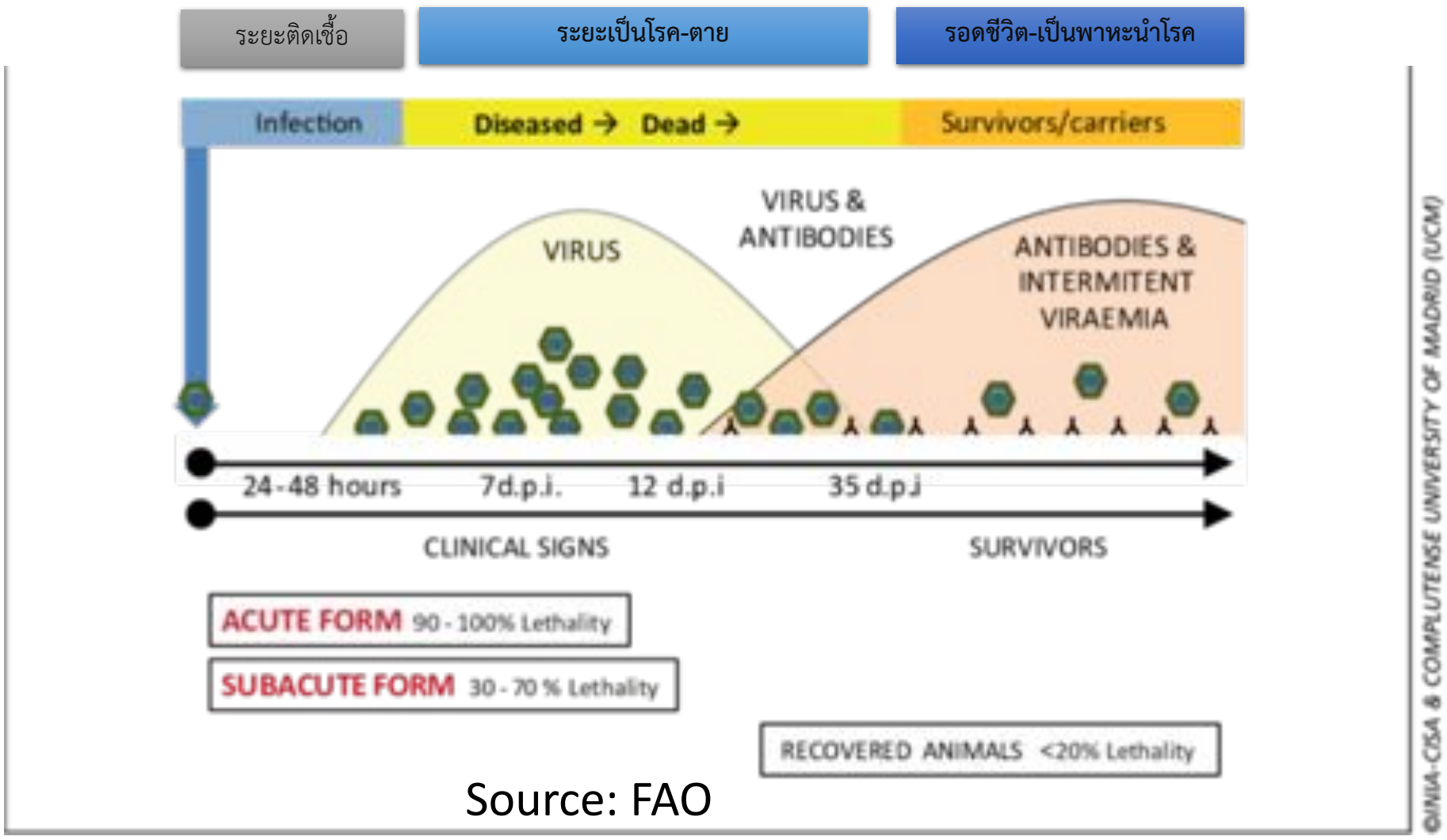


- ☐ PCR
- ☐ ELISA
- ☐ IB/IPT/IFA

☑ non-apparent (carrier)

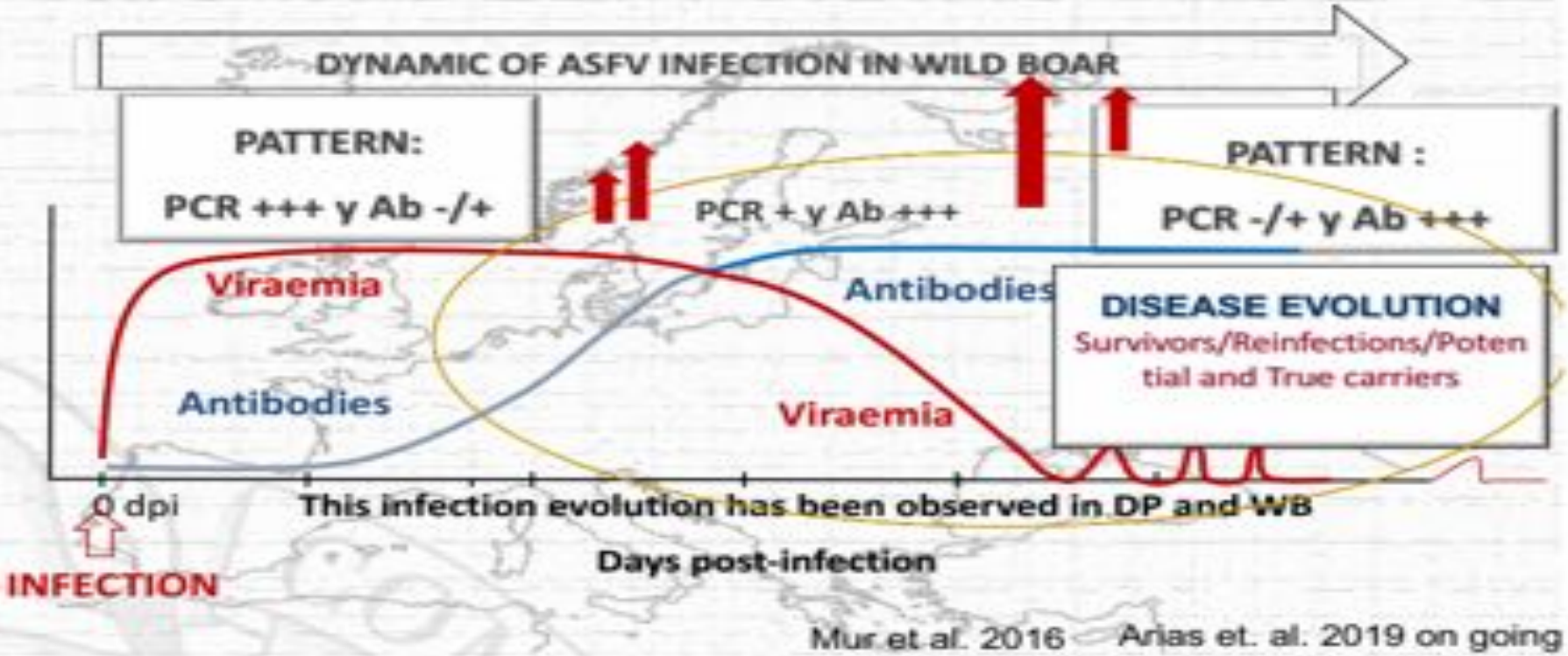
Surveillance programs

Source: Gallardo, 2012


ระยะติดเชื้อ
ระยะเป็นโรค-ตาย
รอดชีวิต-เป็นพาหะนำโรค
Infection
Diseased → Dead →
Survivors/carriers
VIRUS
VIRUS & ANTIBODIES
ANTIBODIES & INTERMITENT VIRAEMIA
24-48 hours
7d.p.i.
12 d.p.i
35 d.p.i
CLINICAL SIGNS
SURVIVORS
ACUTE FORM 90-100% Lethality
SUBACUTE FORM 30-70 % Lethality
RECOVERED ANIMALS <20% Lethality
©INIA-CISA & COMPLUTENSE UNIVERSITY OF MADRID (UCM)

Source: FAO

Evolution of the ASF infection in WB



This evolution don't look genotypes dependent: Observed in gI and gII

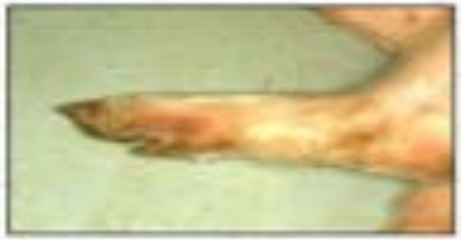
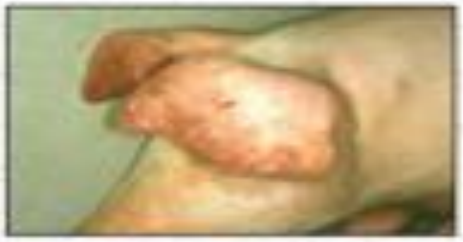
Scientific Conference on ASF
Bangkok, 2019

Prof. JM. Sánchez-Vizcaíno

Acuate-subacuate forms of ASF:

Could be Easily Confused WITH:

- Classical Swine Fever
- Erysipelas
- Salmonellosis
- Actinobacillus (App)
- Other Septicaemic conditions
- PDNS

Thereby laboratory diagnosis is *essential*

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PATHOLOGICAL FINDING WITH ARMENIA 07 IN WB

Tissue	Pathological finding	Percentage of affected animals
Spleen	Splenomegaly	100%
Lymph nodes	Lymphoadenomegaly	94.12%
	Hemorrhagic lymphadenitis	88.23%
Pleura	Hydrotorax	88.23%
Tonsils	Tonsillar hyperemia	88.23%
	Purulent tonsillitis	52.94%
Liver	Hepatomegaly	88.23%
Lung	Shock lung	70.59%
	Bronchiolointerstitial pneumonia	64.7%
Heart	Hydropericardias	70.59%
	Hemorrhages	47%
Intestine	Hemorrhages	76.47%
	Necrotic Peyer's patch	35.29%
Kidney	Hemorrhages	41.17%

Infection by Armenia/07 strain resulted in 100% lethality wild boar (total= 17) in both intramuscular infected (6) and in contact animals (11)

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Bangkok, 2019

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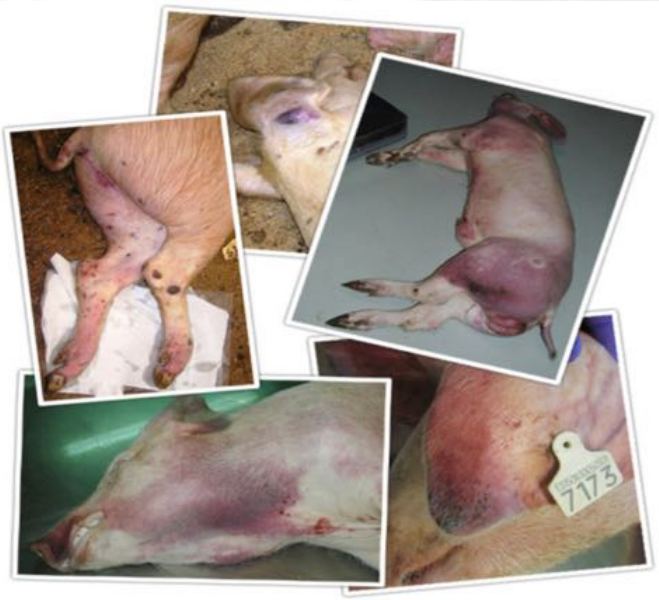
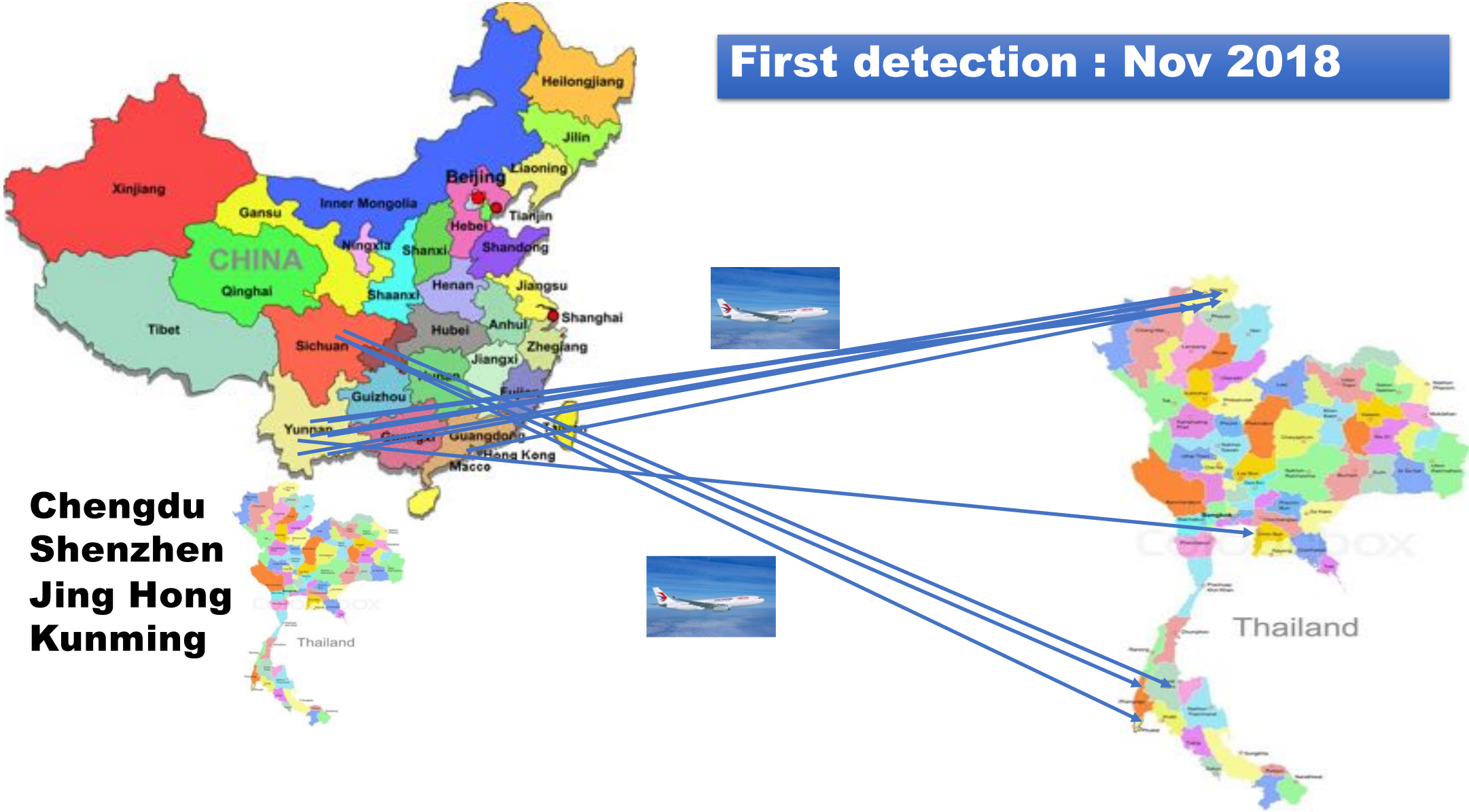
Target samples for ASF diagnosis

5.Pork products



First detection : Nov 2018



Chengdu
Shenzhen
Jing Hong
Kunming

หน่วยงาน สสช/ศวพ	ตัวอย่างส่งตรวจสะสม (1 ต.ค. 61 - 17 พ.ค. 62)														
	ผลิตภัณฑ์			ซากสุกร			วัตถุดิบอาหารสัตว์			โรงฆ่า			ฟาร์มสุกร		
	ตรวจ	บวก	%	ตรวจ	บวก	%	ตรวจ	บวก	%	ตรวจ	บวก	%	ตรวจ	บวก	%
1	143	4	2.8	9	0		21	0		0	0		58	0	
2	545	3	0.6	0	0		0	0		0	0		20	0	
3	157	0		68	0		0	0		0	0		124	0	
4	447	7	1.6	0	0		0	0		73	0		31	0	
5	187	23	12	29	0		0	0		0	0		451	0	
6	31	0		92	0		0	0		19	0		117	0	
7	0	0		20	0		8	0		30	0		164	0	
8	124	8	6.5	11	0		0	0		3	0		465	0	
รวม	1634	45	2.8	229	0		29	0		125	0		1430	0	
ตรวจยืนยัน สสช.	45	45	100	0	0		0	0		0	0		0	0	

Detection method of Thailand

การเก็บตัวอย่างส่งตรวจทางห้องปฏิบัติการ

ตัวอย่างส่งตรวจทางห้องปฏิบัติการ

ส่งทันที

ตรวจวินิจฉัยโรค ASF ด้วย qPCR

ตรวจและรายงานผล

สคบ.

สสช.

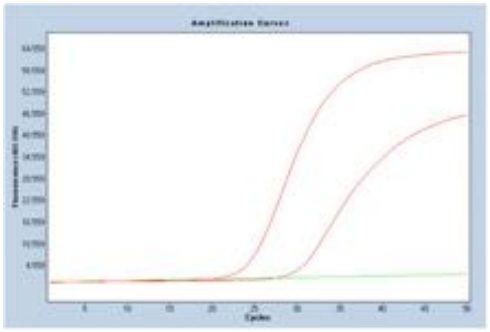
ปศจ.

ปศอ.

ทันที	5 ชม.

การเก็บตัวอย่างส่งตรวจทางห้องปฏิบัติการ

กรณีให้ผลบวก



ส่งตรวจยืนยันที่สสช.

รายงานผล

ศูนย์ปฏิบัติการเผชิญเหตุโรค ASF

12ชม.	72ชม.


ตัวอย่าง
- ซากสุกร: เลือด, ม้าม
- ผลิตภัณฑ์สุกร: แฮม ไส้หมักเกลือ
- วัตถุดิบอาหาร: เนื้อและกระดูกป่น
การเพาะแยกเชื้อ ASF
(Primary cells: PBM)
ผลบวก
ตรวจหาสารพันธุกรรม
เชื้อ ASF ด้วยวิธี qPCR
ผลบวก
การตรวจหาแอนติเจนของ
เชื้อ ASF ด้วย Ag-ELISA
ผลบวก
ตรวจจำแนก genotyping
เชื้อ ASF ด้วยวิธี PCR
ผลบวก
ตรวจหาลำดับ
สารพันธุกรรมของเชื้อ ASF
กรณีผลการทดสอบเป็นลบ ให้ทำการตรวจวินิจฉัยแยกโรค
- โรคอหิวาต์สุกร
- โรคพีอาร์อาร์เอส
- โรคไข้หนังแดง
- โรคซัลโมเนลโลซัส

Screening method

ELSEVIER

Journal of Virological Methods 107 (2003) 53–61

Journal of Virological Methods

www.elsevier.com/locate/jviromet

Development of a TaqMan® PCR assay with internal amplification control for the detection of African swine fever virus

Donald P. King [a,*], Scott M. Reid [b], Geoffrey H. Hutchings [b], Sylvia S. Grierson [a], Philip J. Wilkinson [b], Linda K. Dixon [b], Armanda D.S. Bastos [c], Trevor W. Drew [a]

[a] *Virology Department, Veterinary Laboratories Agency, Woodham Lane, New Haw, Addlestone, Surrey KT15 3NB, UK*
[b] *Pirbright Laboratory, Institute for Animal Health, Ash Road, Pirbright, Woking, Surrey GU24 0NF, UK*
[c] *Department of Zoology and Entomology, Mammal Research Institute, University of Pretoria, Pretoria 0002, South Africa*

Received 19 June 2002; received in revised form 6 August 2002; accepted 7 August 2002

NB: Version adopted by the World Assembly of Delegates of the OIE in May 2012

SECTION 2.8.

SUIDAE

CHAPTER 2.8.1.

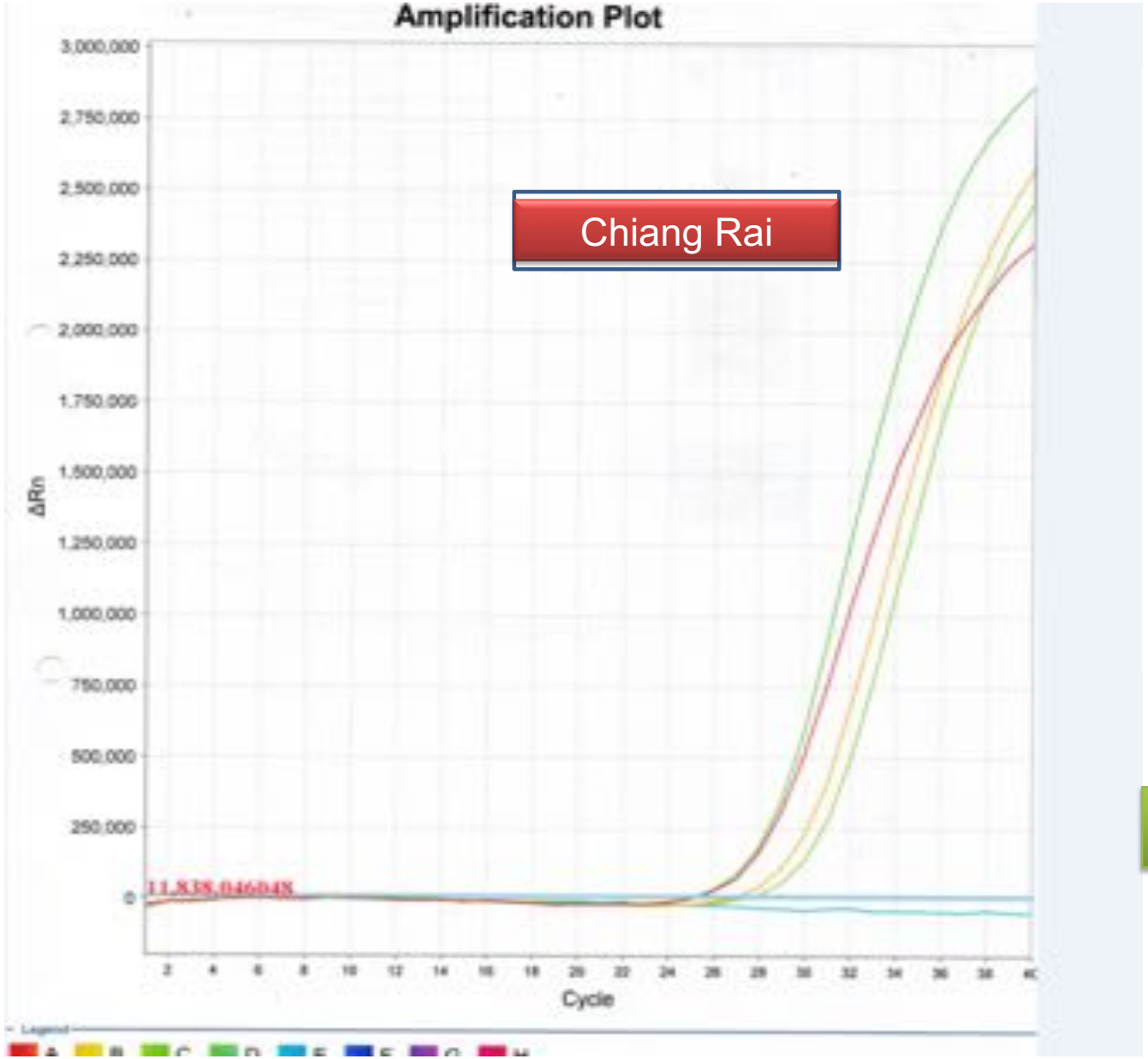
AFRICAN SWINE FEVER

Primer and Probe (King *et al*,2003)

ASF Forward	5'-CTGCT-CATGG-TATCA-ATCTT-ATCGA-3'
ASF Reward	5'-GATAC-CACAA-GATC(AG)- GCCGT-3'
ASFV Probe	[FAM)]- CCACG-GGAGG-AATAC-CAACC-CAGTG-3'-TAMRA

Product size = 250bp

Confirmatory method



Primer and Probe (King *et al*,2003)

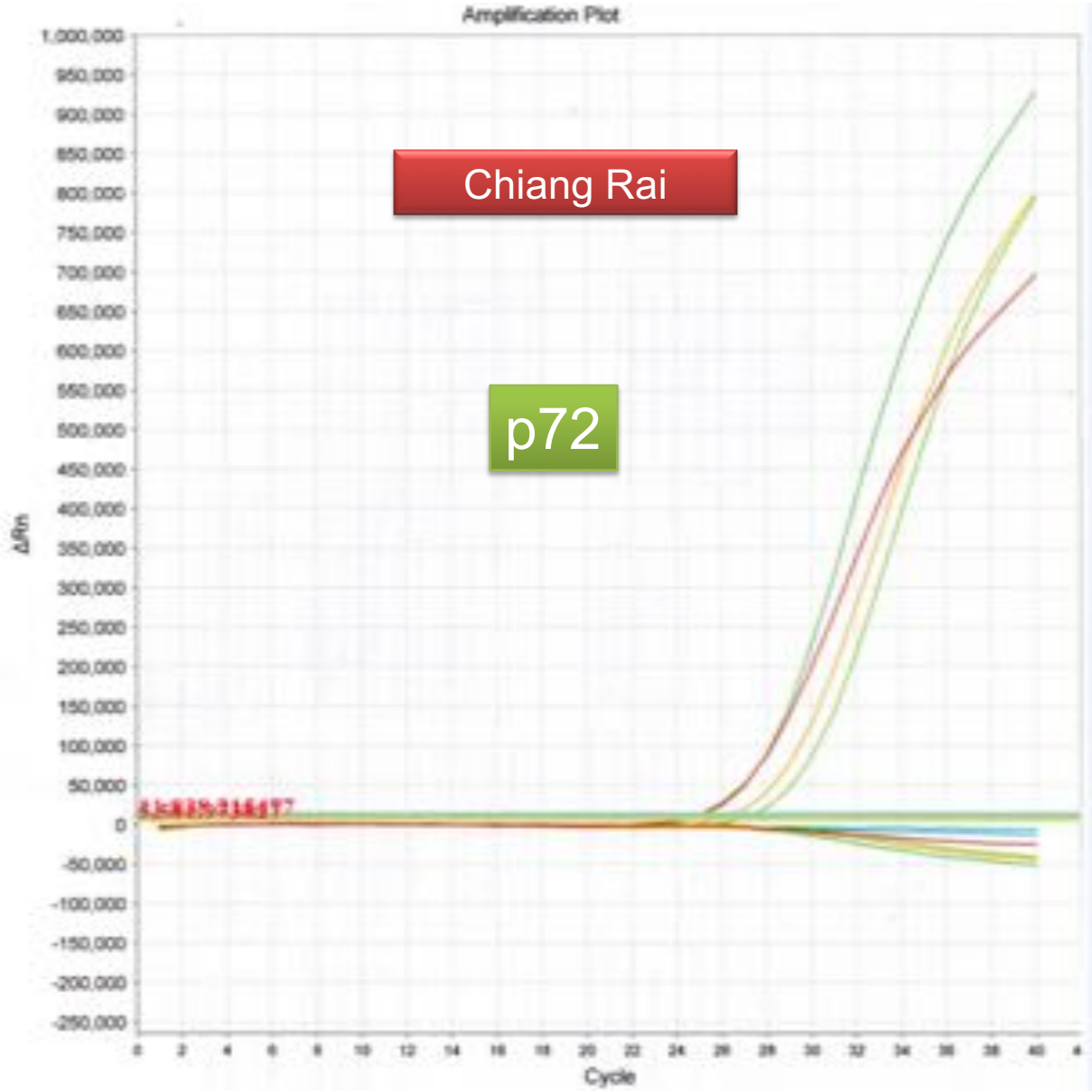
ASF Forward	5'-CTGCT-CATGG-TATCA-ATCTT-ATCGA-3'
ASF Reward	5'-GATAC-CACAA-GATC(AG)- GCCGT-3'
ASFV Probe	[FAM)]- CCACG-GGAGG-AATAC-CAACC-CAGTG-3'-TAMRA

B646L gene

Product size = 250bp

p72

African swine fever-SY18



Transboundary and Emerging Diseases

Transboundary and Emerging Disease

ORIGINAL ARTICLE

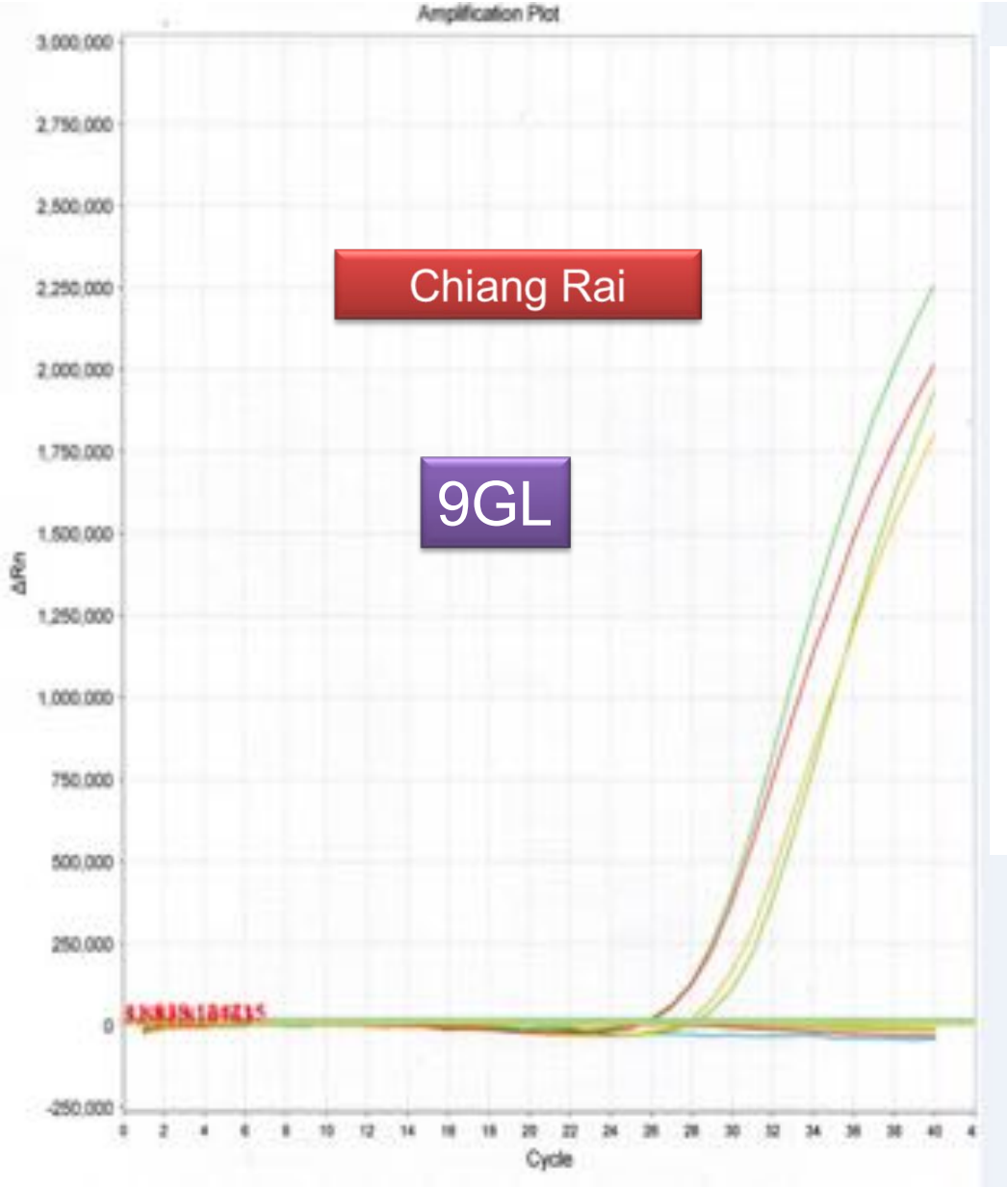
Molecular Diagnosis of African Swine Fever by a New Real-Time PCR Using Universal Probe Library

J. Fernández-Pinero[1], C. Gallardo[1], M. Elizalde[1], A. Robles[1], C. Gómez[1], R. Bishop[2], L. Heath[3], E. Couacy-Hymann[4], F. O. Fasina[5], V. Pelayo[1], A. Soler[1] and M. Arias[1]

[1] Centro de Investigación en Sanidad Animal (CISA-INIA), Madrid, Spain

[2] International Livestock Research Institute (ILRI), Nairobi, Kenya

[3] ARC-Onderstepoort Veterinary Institute, Transboundary Animal Diseases Programme, Pretoria, South Africa



Journal of Virological Methods 168 (2010) 141–146

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Protocols

Sensitive detection of African swine fever virus using real-time PCR with a 5′ conjugated minor groove binder probe

John McKillen[a,*], Michael McMenamy[b], Bernt Hjertner[b], Francis McNeilly[a], Åse Uttenthal[c], Carmina Gallardo[d], Brian Adair[a], Gordon Allan[a]

[a] Veterinary Sciences Division, Agri-Food and Biosciences Institute, Stormont, Belfast BT4 3SD, United Kingdom
[b] Department of Veterinary Science, Queen's University of Belfast, Stormont, Belfast BT4 3SD, United Kingdom
[c] Department of Virology, National Veterinary Institute, DTU, Lindholm, DK-4771 Kalvehave, Denmark
[d] Centro de Investigación en Sanidad Animal (CISA-INIA), Valdeolmos, Madrid 28130, Spain

CENTRO DE INVESTIGACION EN SANIDAD ANIMAL (CISA-INIA)

European Union Reference Laboratory for ASF, (EURL-ASF)
Centro de Investigación en Sanidad Animal
CISA-INIA, Valdeolmos 28130, Madrid, Spain

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SOP/CISA/ASF/GENOTYPING/1

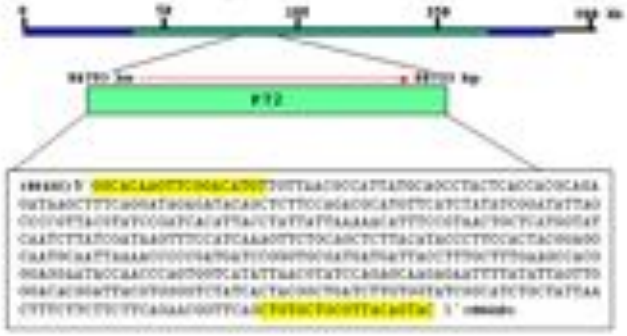
STANDARD OPERATING PROCEDURE FOR GENOTYPING OF AFRICAN SWINE FEVER VIRUS (ASFV) ISOLATES

CONTENTS

1.	PURPOSE.	
2.	SCOPE.	
3.	REFERENCES.	
	3.1.	DOCUMENTS USED IN THE PROCEDURE REDACTION.
	3.2.	COMPLEMENTARY DOCUMENTS (SOPs) TO BE USED.
4.	BACKGROUND INFORMATION.	
5.	PROCEDURE DESCRIPTION.	
	5.1.	EQUIPMENT AND MATERIALS.
	5.2.	PREPARATION.
	5.3.	METHODS.
	5.4.	ANALYSIS AND INTERPRETATION OF RESULTS.
	5.5.	CRITICAL POINTS.
	5.6.	SECURITY MEASURES.
	5.7.	QUALITY CONTROL.

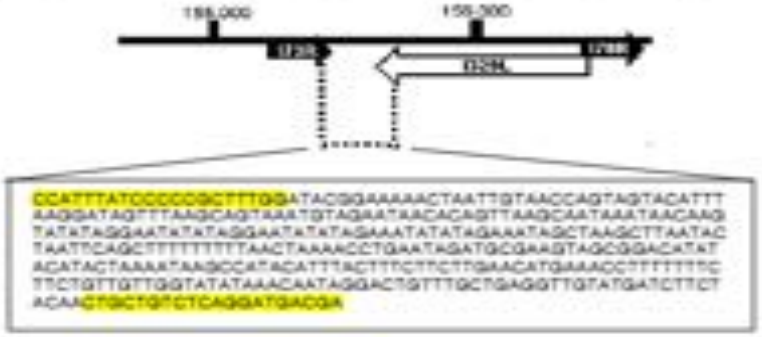
A. **PCR amplification of the C-terminal region of p72 protein using primers p72- U and p72-D.** These primers amplify 478 bp from the protein p72 of the Ba71V ASFV isolate (*GenBank accession no. ASU18466*- Figure 1) and have been previously described by Bastos et al., 2003.

Fig. 1: Sequence obtained using p72U/D primers set (marked in yellow) inside P72 protein.



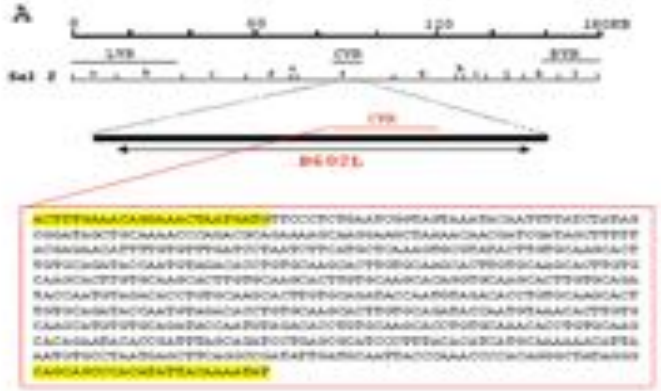
B. **PCR amplification of the intergenic region located between the I73R and I329L genes.** These primers amplify 356 bp located between the I73R and I329L genes and characterized by the presence of TRS of the Gergia ASFV isolate (*GenBank accession no. FR682468.1*- Figure 2) and have been previously described by Gallardo *et* al., 2014).

Fig. 2. Sequence obtained using EcoIA/B primers set (marked in yellow) in the Gergia ASFV strain.



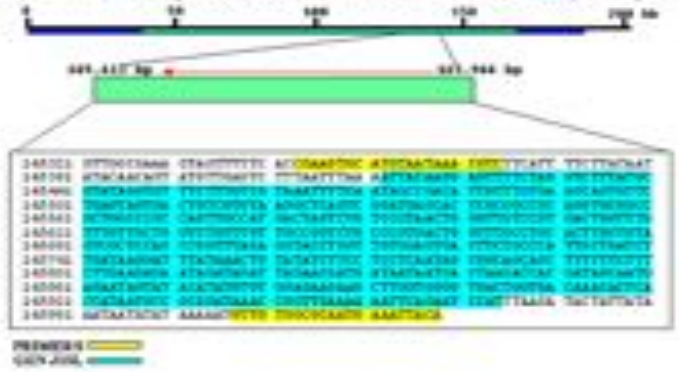
C. **PCR amplification of the CVR within the *B602L* gen using the primer set CVR1 and CVR2.** These primers amplify 665 bp of the Ba71V ASFV isolate (*GenBank accession no. ASU18466*- Figure 3) containing the amino acid tandem repeats and have been previously described by Gallardo *et* al., 2011.

Fig. 3: Sequence obtained after PCR amplification of primers set ORF9L/9F (marked in yellow) within B602L gene.

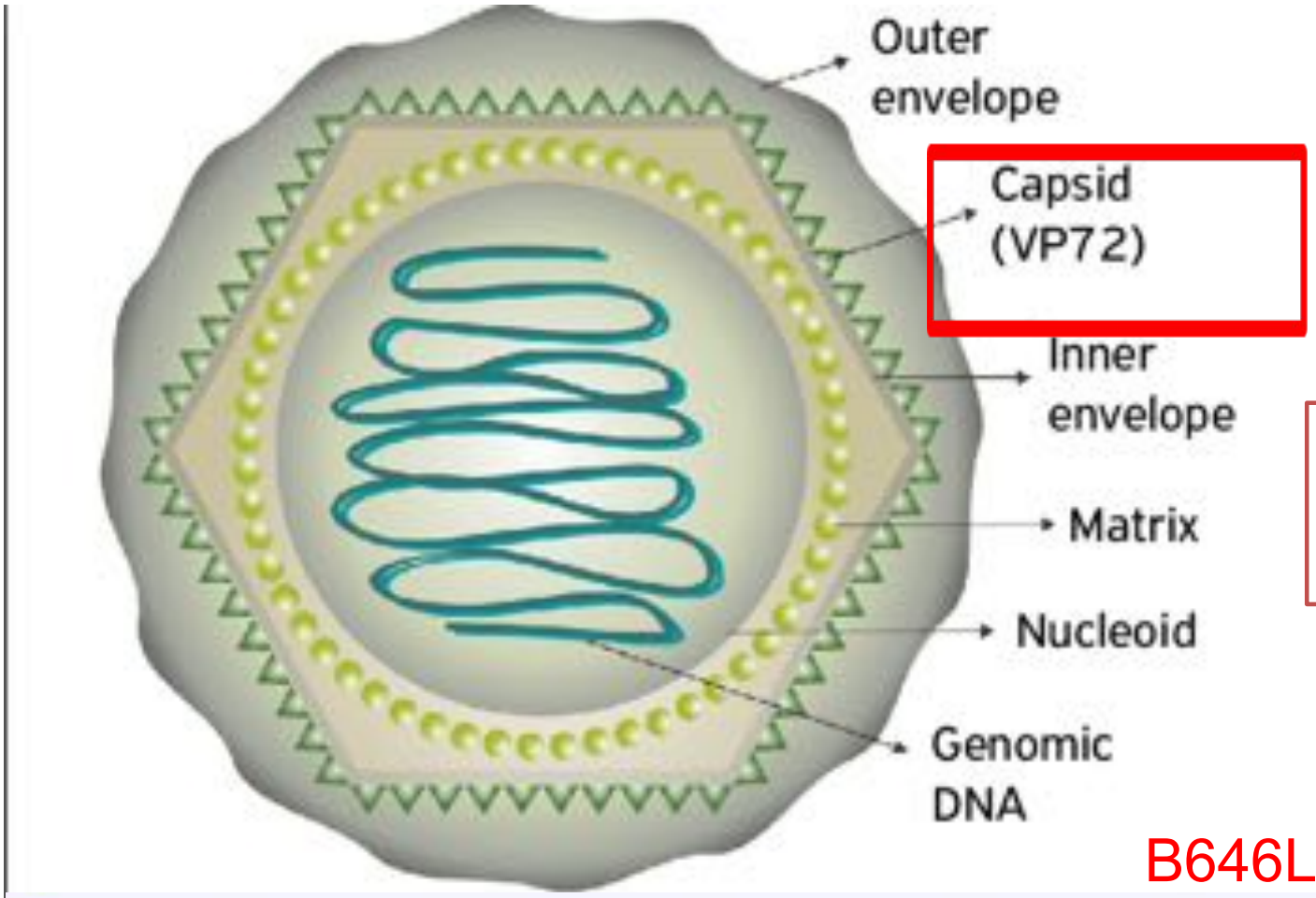


D. **PCR amplification of the full *E183L*-gene encoding the p54 protein using primers PPA89 and PPA722.** These primers amplify 676 bp flanking the complete VP54 sequence of the Ba71V ASFV isolate (*GenBank accession no. ASU18466*- Figure 4) and have been previously described by Gallardo *et* al., 2009.

Fig. 4: Sequence obtained using ASFB9/722 primers set (marked in yellow) flanking the P54 protein

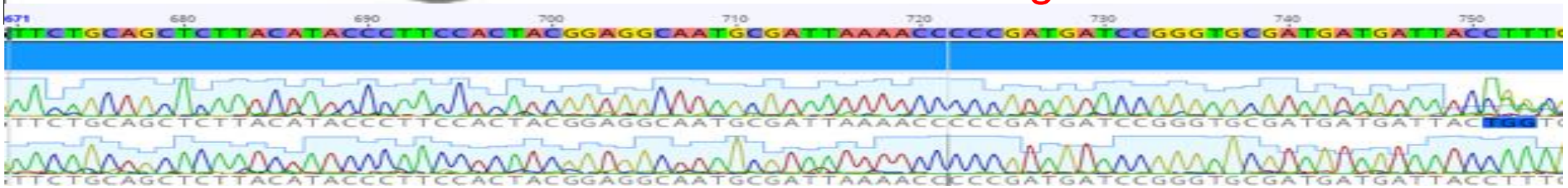


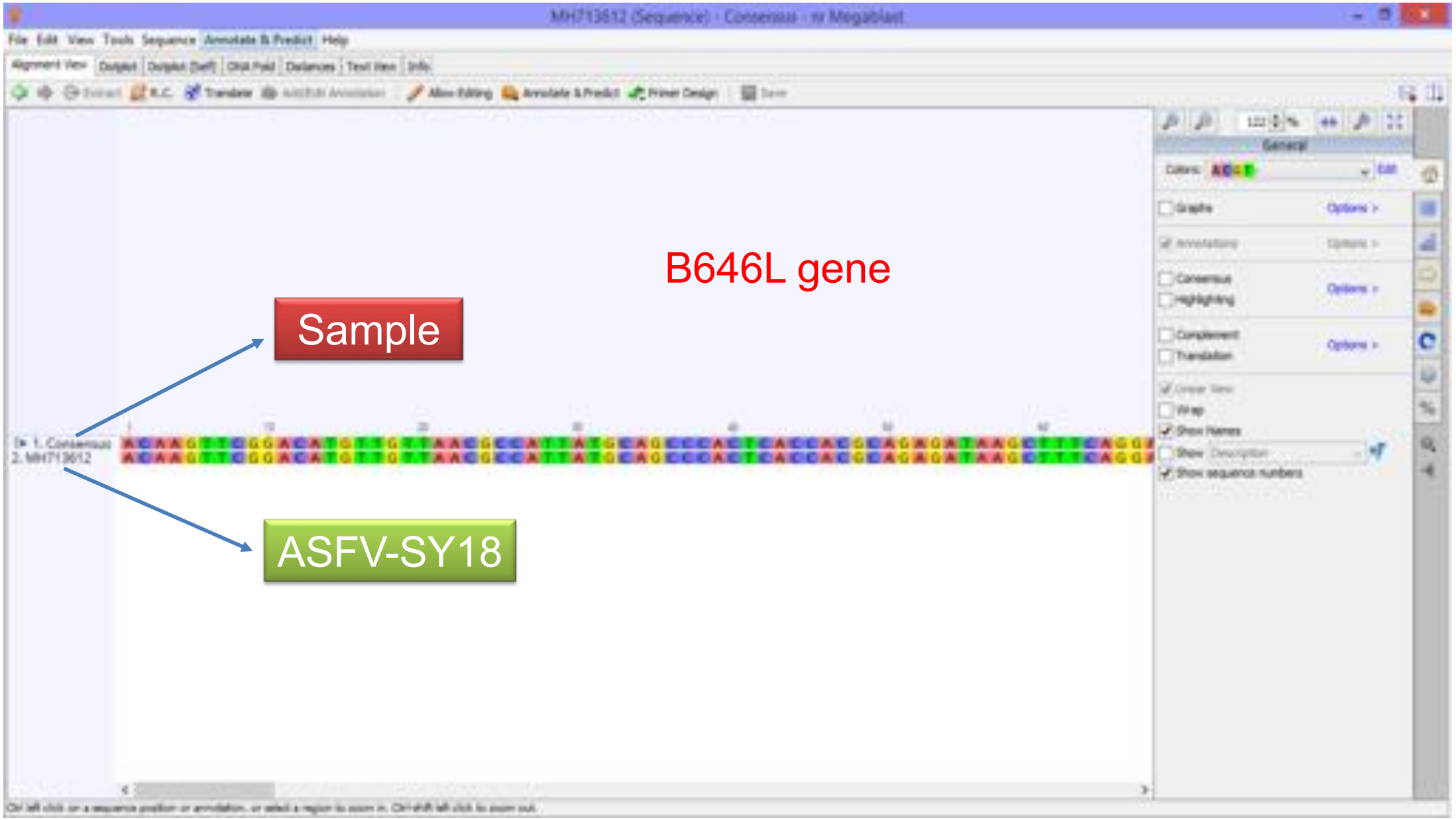
Freitas; Tavares 2016

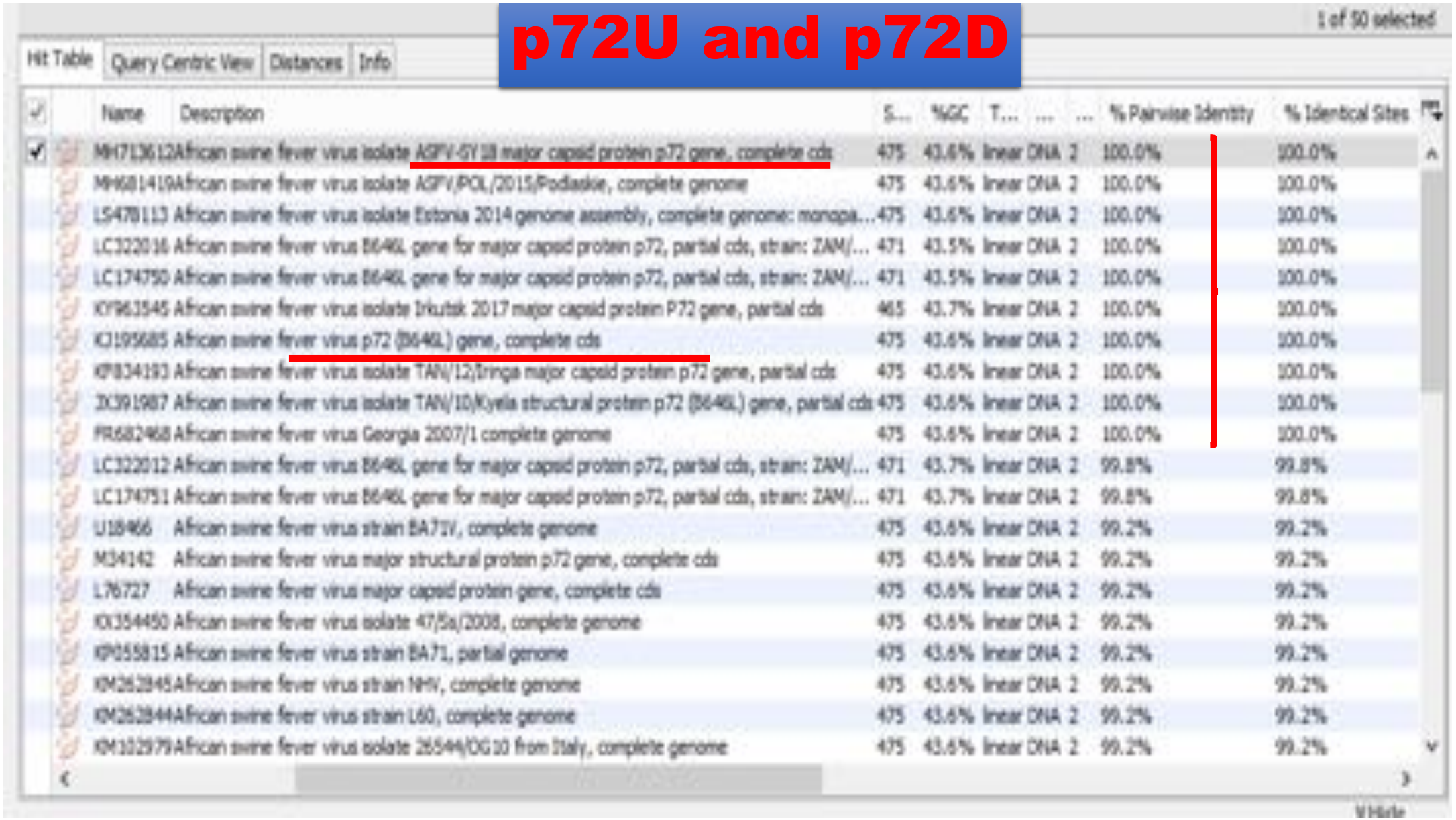


The ASFV genome is made up of double-stranded DNA of between 170 and 190 kb and contains at least 150 genes (Dixon et al., 2000)

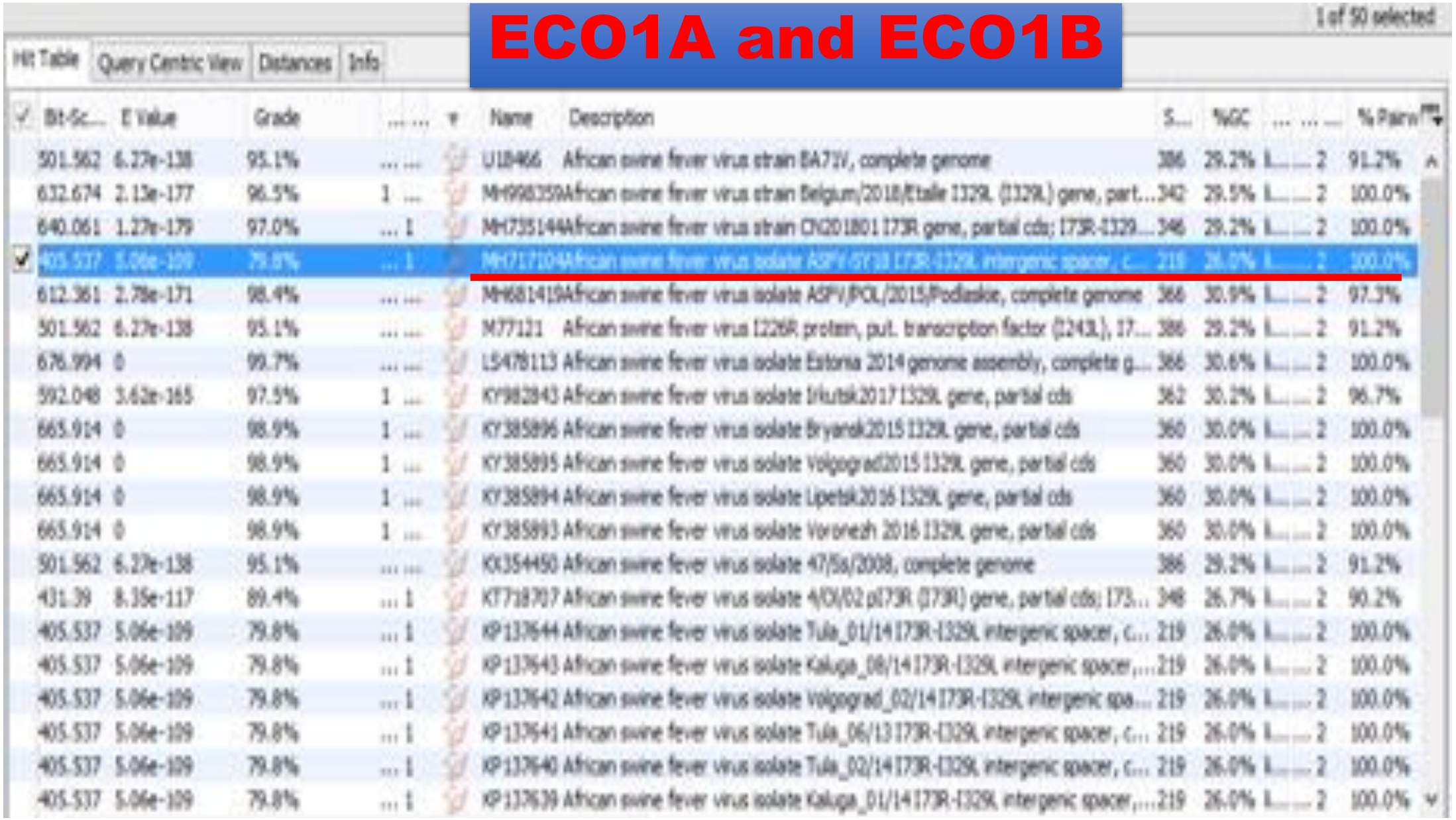
B646L gene




B646L gene
Sample
ASFV-SY18


p72U and p72D
1 of 50 selected
Hit Table
Query Centric View
Distances
Info
Name
Description
S...
%GC
T...
...
...
% Pairwise Identity
% Identical Sites
MH713612 African swine fever virus isolate ASFV-SY18 major capsid protein p72 gene, complete cds 475 43.6% linear DNA 2 100.0% 100.0%
MH681419 African swine fever virus isolate ASFV/POL/2015/Podlaskie, complete genome 475 43.6% linear DNA 2 100.0% 100.0%
LS478113 African swine fever virus isolate Estonia 2014 genome assembly, complete genome: monopa... 475 43.6% linear DNA 2 100.0% 100.0%
LC322016 African swine fever virus B646L gene for major capsid protein p72, partial cds, strain: ZAM/... 471 43.5% linear DNA 2 100.0% 100.0%
LC174750 African swine fever virus B646L gene for major capsid protein p72, partial cds, strain: ZAM/... 471 43.5% linear DNA 2 100.0% 100.0%
KY963545 African swine fever virus isolate Irkutsk 2017 major capsid protein P72 gene, partial cds 465 43.7% linear DNA 2 100.0% 100.0%
KJ195685 African swine fever virus p72 (B646L) gene, complete cds 475 43.6% linear DNA 2 100.0% 100.0%
KP834193 African swine fever virus isolate TAN/12/Iringa major capsid protein p72 gene, partial cds 475 43.6% linear DNA 2 100.0% 100.0%
IX391987 African swine fever virus isolate TAN/10/Kyela structural protein p72 (B646L) gene, partial cds 475 43.6% linear DNA 2 100.0% 100.0%
FR682468 African swine fever virus Georgia 2007/1 complete genome 475 43.6% linear DNA 2 100.0% 100.0%
LC322012 African swine fever virus B646L gene for major capsid protein p72, partial cds, strain: ZAM/... 471 43.7% linear DNA 2 99.8% 99.8%
LC174751 African swine fever virus B646L gene for major capsid protein p72, partial cds, strain: ZAM/... 471 43.7% linear DNA 2 99.8% 99.8%
U18466 African swine fever virus strain BA71V, complete genome 475 43.6% linear DNA 2 99.2% 99.2%
M34142 African swine fever virus major structural protein p72 gene, complete cds 475 43.6% linear DNA 2 99.2% 99.2%
L76727 African swine fever virus major capsid protein gene, complete cds 475 43.6% linear DNA 2 99.2% 99.2%
KX354450 African swine fever virus isolate 47/Sa/2008, complete genome 475 43.6% linear DNA 2 99.2% 99.2%
KP055815 African swine fever virus strain BA71, partial genome 475 43.6% linear DNA 2 99.2% 99.2%
KM262845 African swine fever virus strain NH/, complete genome 475 43.6% linear DNA 2 99.2% 99.2%
KM262844 African swine fever virus strain L60, complete genome 475 43.6% linear DNA 2 99.2% 99.2%
KM102979 African swine fever virus isolate 26544/OG10 from Italy, complete genome 475 43.6% linear DNA 2 99.2% 99.2%

ECO1A and ECO1B



1 of 50 selected

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	632.674	2.13e-177	96.5%	1 ...	MH998359	African swine fever virus strain Belgium/2018/Etaile I329L (I329L) gene, part...	342	29.5%	L... ... 2	100.0%
	640.061	1.23e-179	97.0%	... 1	MH735144	African swine fever virus strain CN/GD/1801 I73R gene, partial cds; I73R-I329...	346	29.2%	L... ... 2	100.0%
✓	405.537	5.06e-109	79.8%	... 1	MH717104	African swine fever virus isolate ASFV/SY/18 I73R-I329L intergenic spacer, c...	219	26.0%	L... ... 2	100.0%
	612.361	2.78e-171	98.4%	... ...	MH681419	African swine fever virus isolate ASFV/POL/2015/Podlaskie, complete genome	366	30.9%	L... ... 2	97.3%
	501.562	6.27e-138	95.1%	... ...	M77121	African swine fever virus I226R protein, put. transcription factor (I243L), 17...	386	29.2%	L... ... 2	91.2%
	676.994	0	99.7%	... ...	LS478113	African swine fever virus isolate Estonia 2014 genome assembly, complete g...	366	30.6%	L... ... 2	100.0%
	592.048	3.62e-165	97.5%	1 ...	KY982843	African swine fever virus isolate Irkutsk2017 I329L gene, partial cds	362	30.2%	L... ... 2	96.7%
	665.914	0	98.9%	1 ...	KY385896	African swine fever virus isolate Bryansk2015 I329L gene, partial cds	360	30.0%	L... ... 2	100.0%
	665.914	0	98.9%	1 ...	KY385895	African swine fever virus isolate Volgograd2015 I329L gene, partial cds	360	30.0%	L... ... 2	100.0%
	665.914	0	98.9%	1 ...	KY385894	African swine fever virus isolate Lipetsk2016 I329L gene, partial cds	360	30.0%	L... ... 2	100.0%
	665.914	0	98.9%	1 ...	KY385893	African swine fever virus isolate Voronezh 2016 I329L gene, partial cds	360	30.0%	L... ... 2	100.0%
	501.562	6.27e-138	95.1%	... ...	KX354450	African swine fever virus isolate 47/Ss/2008, complete genome	386	29.2%	L... ... 2	91.2%
	431.39	8.35e-117	89.4%	... 1	KT718707	African swine fever virus isolate 4/OI/02 p73R (I73R) gene, partial cds; I73...	348	26.7%	L... ... 2	90.2%
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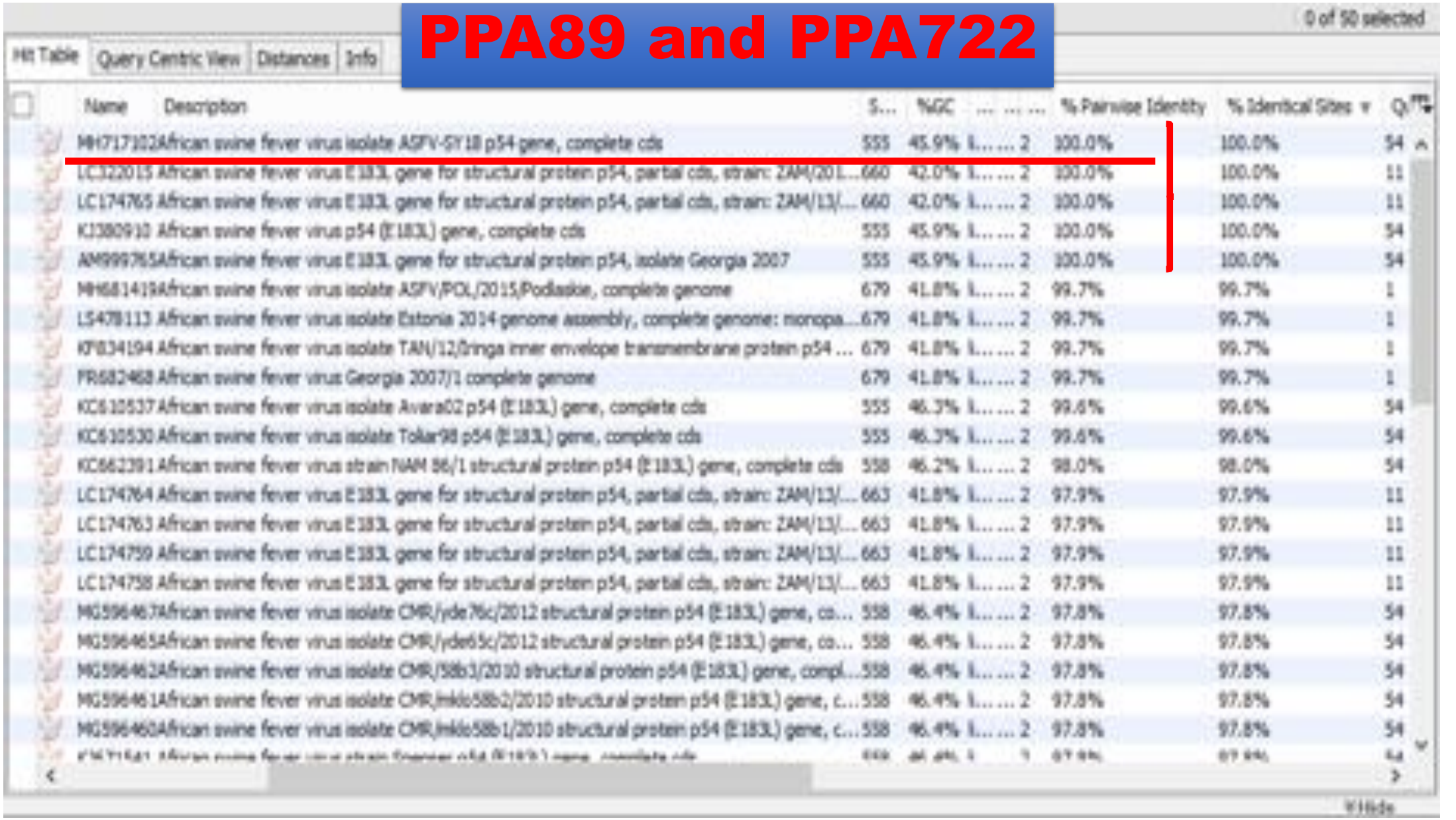
PPA89 and PPA722
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% Pairwise Identity
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MH717102 African swine fever virus isolate ASFV-SY18 p54 gene, complete cds 555 45.9% 100.0% 100.0% 54
LC322015 African swine fever virus E183L gene for structural protein p54, partial cds, strain: ZAM/201... 660 42.0% 100.0% 100.0% 11
LC174765 African swine fever virus E183L gene for structural protein p54, partial cds, strain: ZAM/13/... 660 42.0% 100.0% 100.0% 11
KJ380910 African swine fever virus p54 (E183L) gene, complete cds 555 45.9% 100.0% 100.0% 54
AM999765 African swine fever virus E183L gene for structural protein p54, isolate Georgia 2007 555 45.9% 100.0% 100.0% 54
MH681419 African swine fever virus isolate ASFV/POL/2015/Podlaskie, complete genome 679 41.8% 99.7% 99.7% 1
LS478113 African swine fever virus isolate Estonia 2014 genome assembly, complete genome: monopa... 679 41.8% 99.7% 99.7% 1
KP834194 African swine fever virus isolate TAN/12/Iringa inner envelope transmembrane protein p54 ... 679 41.8% 99.7% 99.7% 1
FR682468 African swine fever virus Georgia 2007/1 complete genome 679 41.8% 99.7% 99.7% 1
KC610537 African swine fever virus isolate Avara02 p54 (E183L) gene, complete cds 555 46.3% 99.6% 99.6% 54
KC610530 African swine fever virus isolate Tolar98 p54 (E183L) gene, complete cds 555 46.3% 99.6% 99.6% 54
KC662391 African swine fever virus strain NAM 86/1 structural protein p54 (E183L) gene, complete cds 558 46.2% 98.0% 98.0% 54
LC174764 African swine fever virus E183L gene for structural protein p54, partial cds, strain: ZAM/13/... 663 41.8% 97.9% 97.9% 11
LC174763 African swine fever virus E183L gene for structural protein p54, partial cds, strain: ZAM/13/... 663 41.8% 97.9% 97.9% 11
LC174759 African swine fever virus E183L gene for structural protein p54, partial cds, strain: ZAM/13/... 663 41.8% 97.9% 97.9% 11
LC174758 African swine fever virus E183L gene for structural protein p54, partial cds, strain: ZAM/13/... 663 41.8% 97.9% 97.9% 11
MG596467 African swine fever virus isolate CMR/yde76c/2012 structural protein p54 (E183L) gene, co... 558 46.4% 97.8% 97.8% 54
MG596465 African swine fever virus isolate CMR/yde65c/2012 structural protein p54 (E183L) gene, co... 558 46.4% 97.8% 97.8% 54
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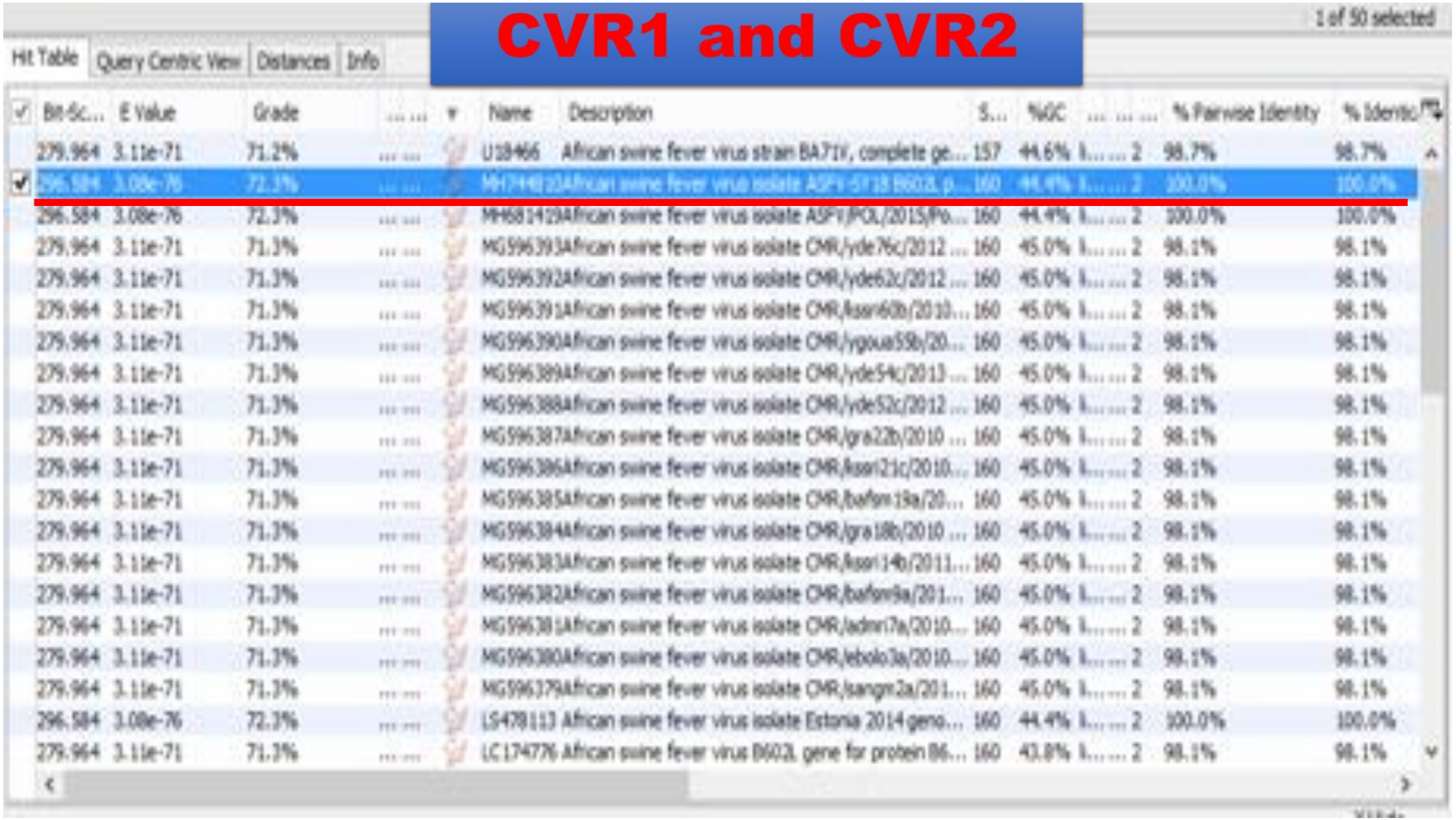
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Name
Description
S...
%GC
% Pairwise Identity
% Identic
279.964 3.11e-71 71.2% U18466 African swine fever virus strain BA71V, complete ge... 157 44.6% 98.7% 98.7%
296.584 3.08e-76 72.3% MH744810African swine fever virus isolate ASFV-5718 B602L p... 160 44.4% 100.0% 100.0%
296.584 3.08e-76 72.3% MH681419African swine fever virus isolate ASFV/POL/2015/Po... 160 44.4% 100.0% 100.0%
279.964 3.11e-71 71.3% MG596393African swine fever virus isolate CMR/yde76c/2012 ... 160 45.0% 98.1% 98.1%
279.964 3.11e-71 71.3% MG596392African swine fever virus isolate CMR/yde62c/2012 ... 160 45.0% 98.1% 98.1%
279.964 3.11e-71 71.3% MG596391African swine fever virus isolate CMR/kseri60b/2010... 160 45.0% 98.1% 98.1%
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279.964 3.11e-71 71.3% MG596388African swine fever virus isolate CMR/yde52c/2012 ... 160 45.0% 98.1% 98.1%
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279.964 3.11e-71 71.3% MG596382African swine fever virus isolate CMR/bafon9a/201... 160 45.0% 98.1% 98.1%
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296.584 3.08e-76 72.3% LS478113 African swine fever virus isolate Estonia 2014 geno... 160 44.4% 100.0% 100.0%
279.964 3.11e-71 71.3% LC174776 African swine fever virus B602L gene for protein B6... 160 43.8% 98.1% 98.1%

Molecular Characterization of African Swine Fever Virus, China, 2018

Shengqiang Ge[1], Jinming Li[1], Xiaoxu Fan[1], Fuxiao Liu[1], Lin Li[1], Qinghua Wang, Weijie Ren, Jingyue Bao, Chunju Liu, Hua Wang, Yuntian Liu, Yongqiang Zhang, Tiangang Xu, Xiaodong Wu[2], and Zhiliang Wang[2]

Author affiliations: China Animal Health and Epidemiology Center, Qingdao, China

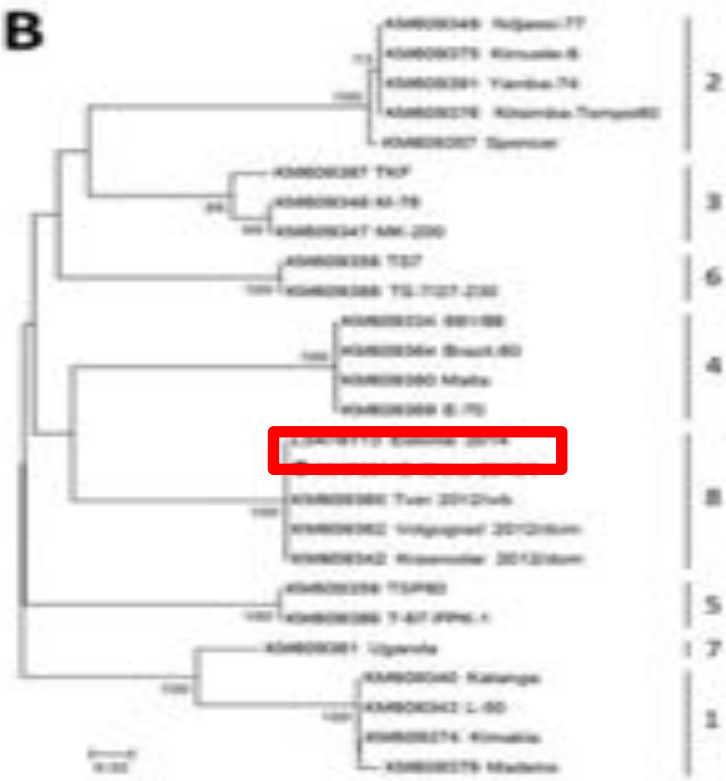
p72

Sequencing primer (Bastos *et al*,2003)

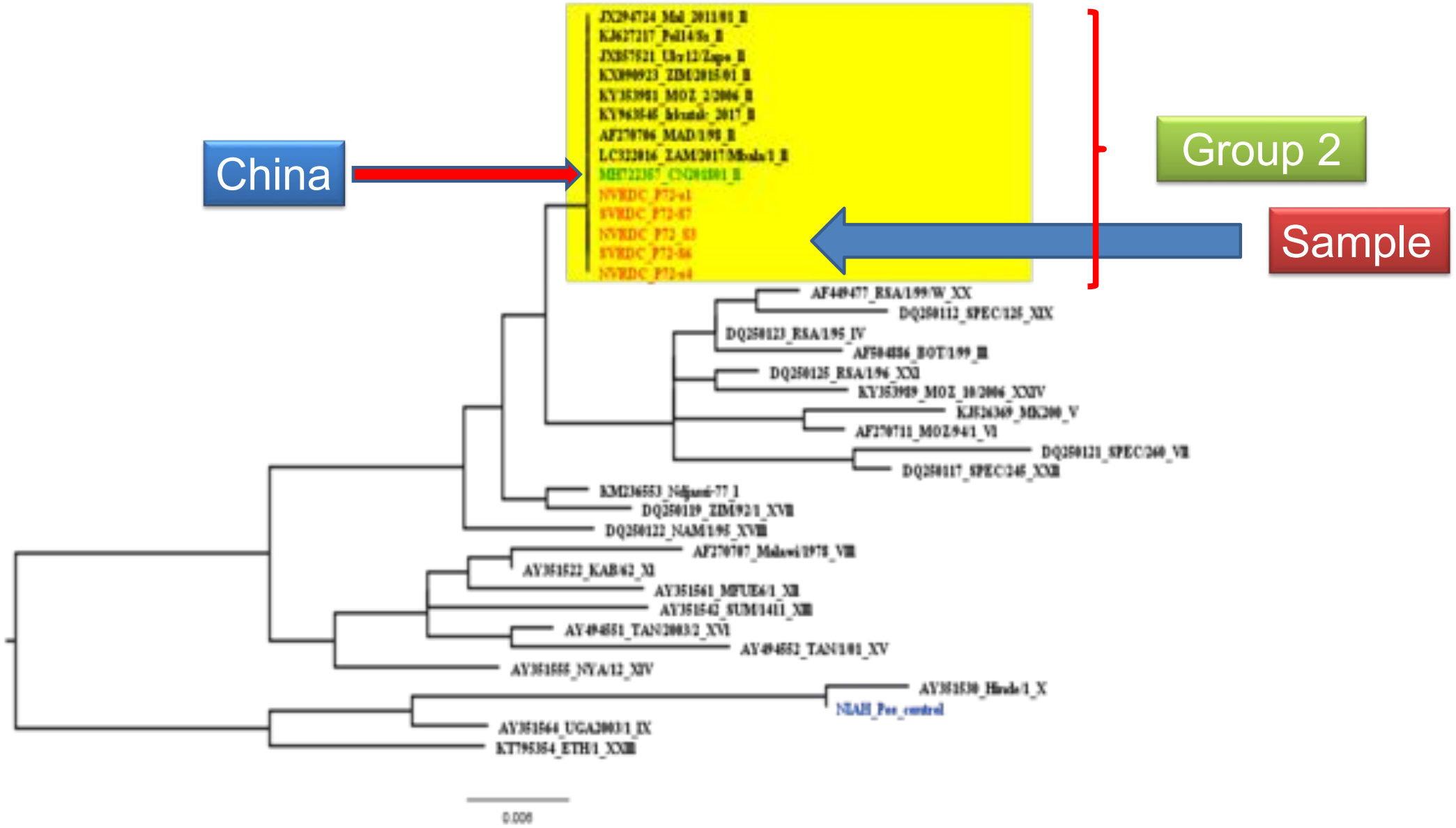
p72-U	5'-GGCACAAGTTCGGACATGT-3'
p72-D	5'-GTACTGTAACGCAGCACAG-3'

Product size = 478bp

B646L gene



EP402R


China
Group 2
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KJ627217_Pol14/Su_II
JX857521_Ukr12/Zapo_II
KX090923_ZIM/2015/01_II
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KY963845_Ivkuzuk-2017_II
AF270706_MAD/1/95_II
LC322016_ZAM/2017/Mbala/1_II
MH722367_CN/2018/1_II
NVRDC_P72-e1
NVRDC_P72-87
NVRDC_P72_83
NVRDC_P72-86
NVRDC_P72-e4
AF449477_RSA/1/99/W_XX
DQ250112_SPEC/125_XIX
DQ250123_RSA/1/95_IV
AF504836_BOT/1/99_III
DQ250125_RSA/1/96_XXI
KY353989_MOZ_10/2006_XXIV
KJ526369_MK200_V
AF270711_MOZ/94/1_VI
DQ250121_SPEC/240_VII
DQ250117_SPEC/245_XXII
KM236553_Ndjamé-77_1
DQ250119_ZIM/92/1_XVII
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AF270707_Malawi/1978_VIII
AY351522_KAB/62_XI
AY351561_MFUE6/1_XII
AY351542_SUM/1411_XIII
AY494651_TAN/2003/2_XVI
AY494652_TAN/1/01_XV
AY351555_NYA/12_XIV
AY351530_Hinde/1_X
NIAH_Pos_control
AY351564_UGA/2003/1_IX
KT795354_ETH/1_XXIII
0.006


สถาบันสุขภาพสัตว์แห่งชาติ
กรมปศุสัตว์ กระทรวงเกษตรและสหกรณ์
National Institute of Animal Health (NIAH)
ISO 17025 & 9001
DLD Quality Award
English
Journal
ISO 9001 report
Newsletter
ISO 17025 report
Biosafety document
What
PRRS
Spec 2562
Spec 2561
งานการเงินและบัญชี
งานแผนและงบประมาณ
งานโสตทัศนูปกรณ์
งานคอมพิวเตอร์
โรคอหิวาตแอฟริกาในสุกร
Download

IDENTIFICATION OF ANTIBODIES AGAINST ASFV

- OIE ELISA
- IMMUNOBLOTTING
- INDIRECT FLUORESCENT ANTIBODY TEST
- COMMERCIAL ELISA

SECTION 2.8.

SUIDAE

CHAPTER 2.8.1.

AFRICAN SWINE FEVER

Scientific Conference on ASF
Bangkok, 2019

Prof. JM. Sánchez-Vizcaíno

New techniques for ASF diagnosis

PENSIDE TEST

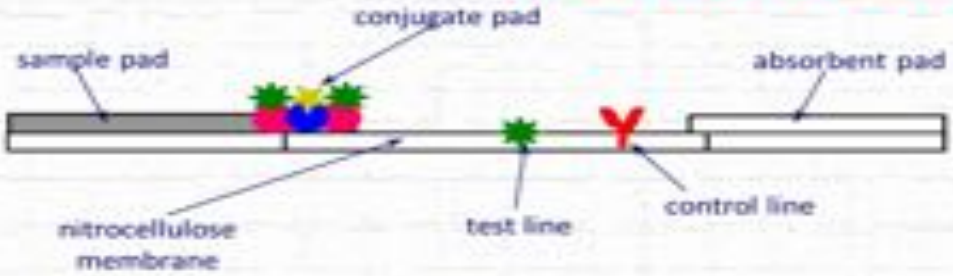
- Direct immunochromatography
- Antigen vp72
- Serum, plasma and full blood
- Tested in DP and WB
- **Field conditions!!**

99% correspondence with the OIE ELISA. 82% sensitivity respect to IPMA (wild boars)

99.9% correspondence with INGEZIM® PPA COMPAC and OIE ELISA. 96% specificity respect IPMA (wild boars)

INGEZIM PPA CROM

TECHNICAL BASIS & PRODUCT APPLICATION



IPMA = IPT

Test Line: VP72 protein of ASFV adsorbed on the nitrocellulose membrane

Control Line: α-control protein MAb adsorbed on the nitrocellulose membrane.

Red latex microparticles covalently coated with VP72 protein.

Blue latex microparticles covalently coated with a control protein.

Scientific Conference on ASF
Bangkok, 2019

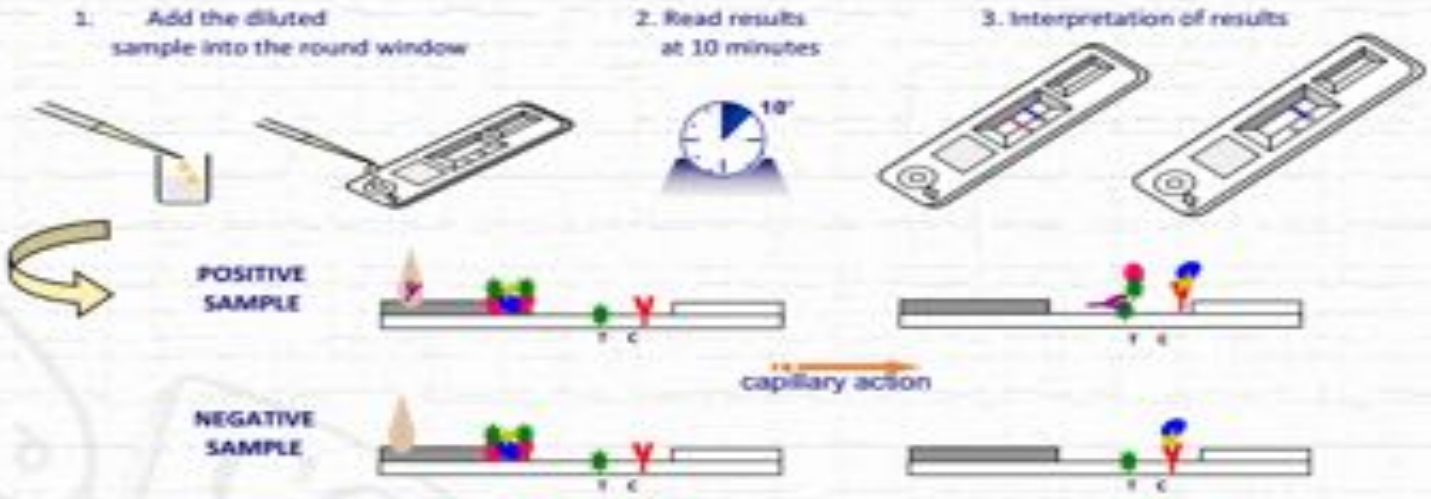
Prof. JM. Sánchez-Vizcaíno

New techniques for ASF diagnosis

PENSIDE TEST

INGEZIM PPA CROM

TEST PROCEDURE



Scientific Conference on ASF
Bangkok, 2019

Prof. JM. Sánchez-Vizcaíno

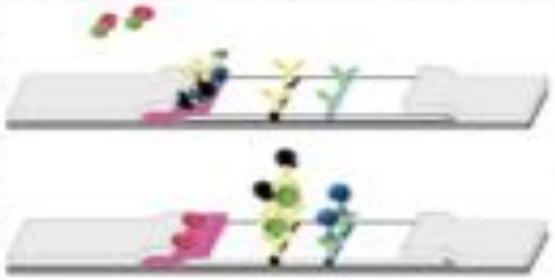
New techniques for ASF diagnosis

PENSIDE TEST

- Direct immunochromatography
- Monoclonal antigen vp72
- Blood
- Tested in DP and WB
- **Field conditions!!**

INGEZIM ASF CROM ANTIGEN

R.11.ASF.K42



the sensitivity of the assay respect to rt-PCR was 76% and the specificity 96%.

Scientific Conference on ASF
Bangkok, 2019

Prof. JM. Sánchez-Vizcaíno

IV. INTERPRETATION OF RESULTS

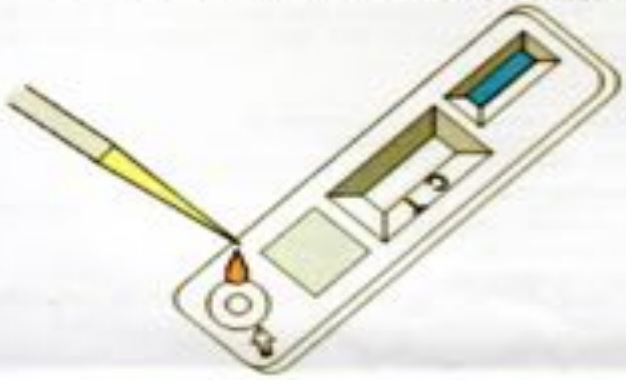
Interpretation of results should be done as follows, 10 minutes after the addition of the sample:

NEGATIVE: A single blue line appears in the result area.

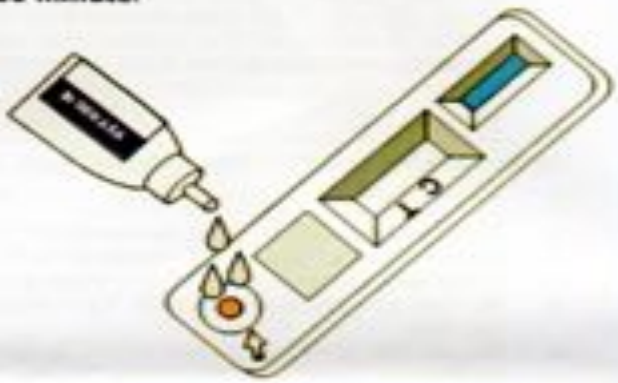
POSITIVE: A blue line and a second black line appear in the result area. Colour intensity may vary depending on the antigen concentration.

INVALID: If no blue line appears, the test will be invalid. Repeat the test using a new device.

- **No results must be considered after 10 minute.**



1. Add 20 µL of sample to the round window



2. Add 3-4 drops of running buffer

POSITIVE

NEGATIVE


CT
CT

Comparison of extraction method

GF-1 Tissue DNA Extraction Kit

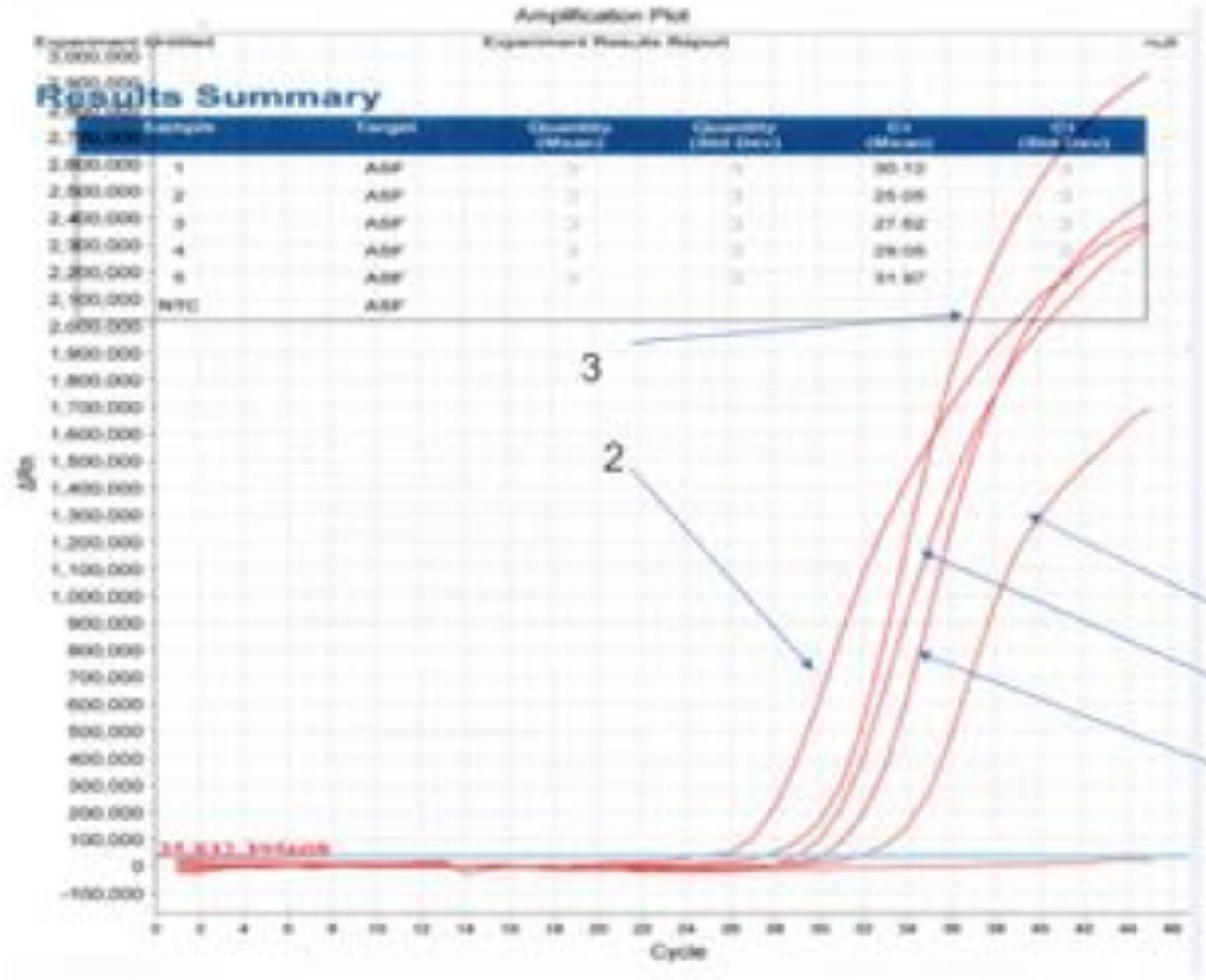
DNeasy mericon Food Kit

MagMAX™ Pathogen RNA/DNA Kit

High Pure PCR Template

QIAamp DNA Mini Kit

Sample extraction



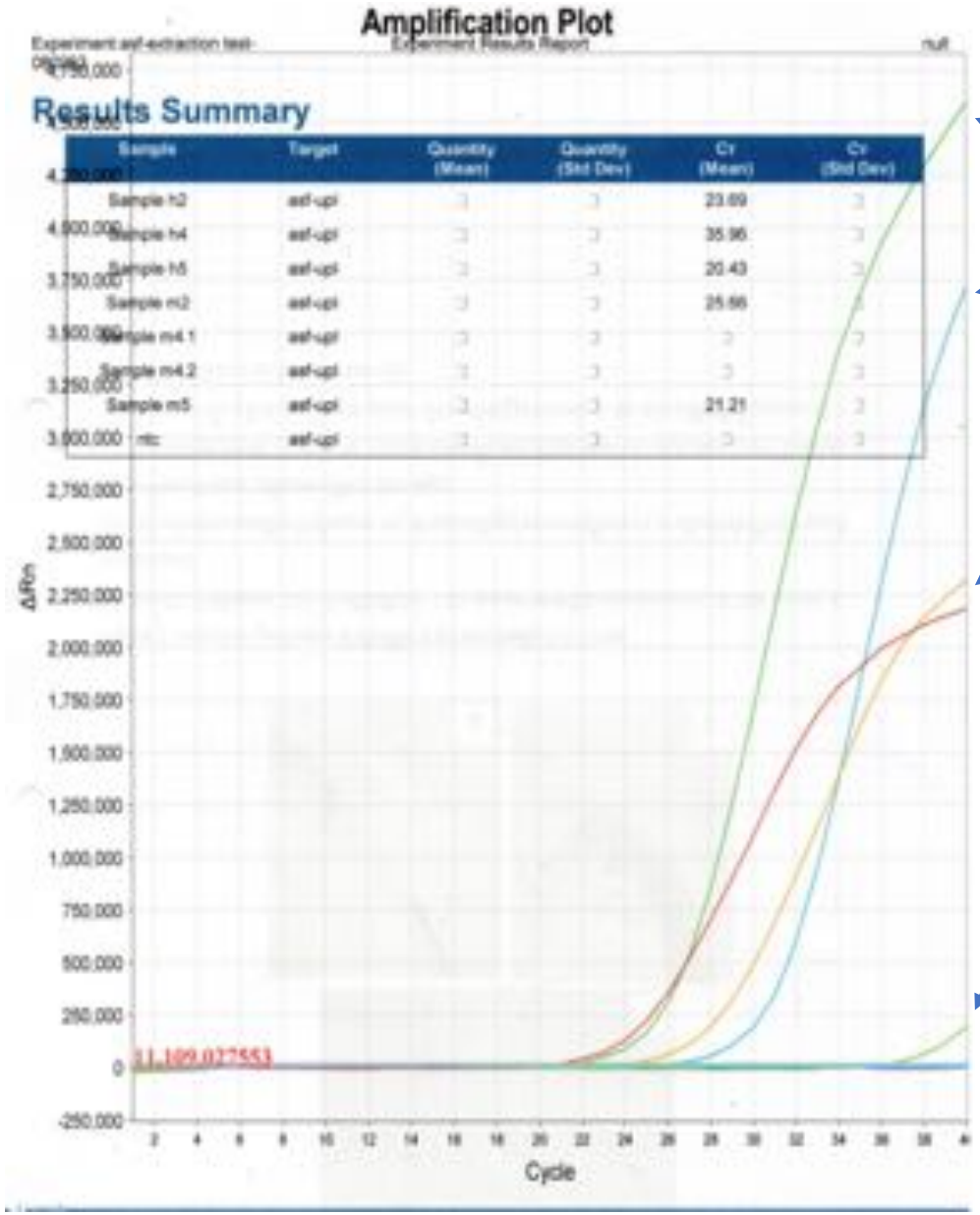
2.High pure: ct 25.05

3.Mericon : ct 27.62

4.GF1: ct 29.05

1.Magmax : ct 30.12

5.Qiagen DNA : ct 31.97



Different sample

m=mericon

h=high pure

Sample No. 2,4,5

Laboratory network and proficiency testing

เครือข่ายทางห้องปฏิบัติการ

สถาบันสุขภาพสัตว์แห่งชาติ

ศูนย์วิจัยและพัฒนาการสัตวแพทย์

จุฬาฯ

ม.เกษตร

ม.มหิดล

ม.เชียงใหม่

ม.ขอนแก่น

ม.มหาสารคาม

ม.สงขลานครินทร์

ห้องปฏิบัติการเอกชน

ด่านกักกันสัตว์

องค์การสวนสัตว์

Diagnostic Tool box and Regional Proficiency Testing

David Williams and John Allen

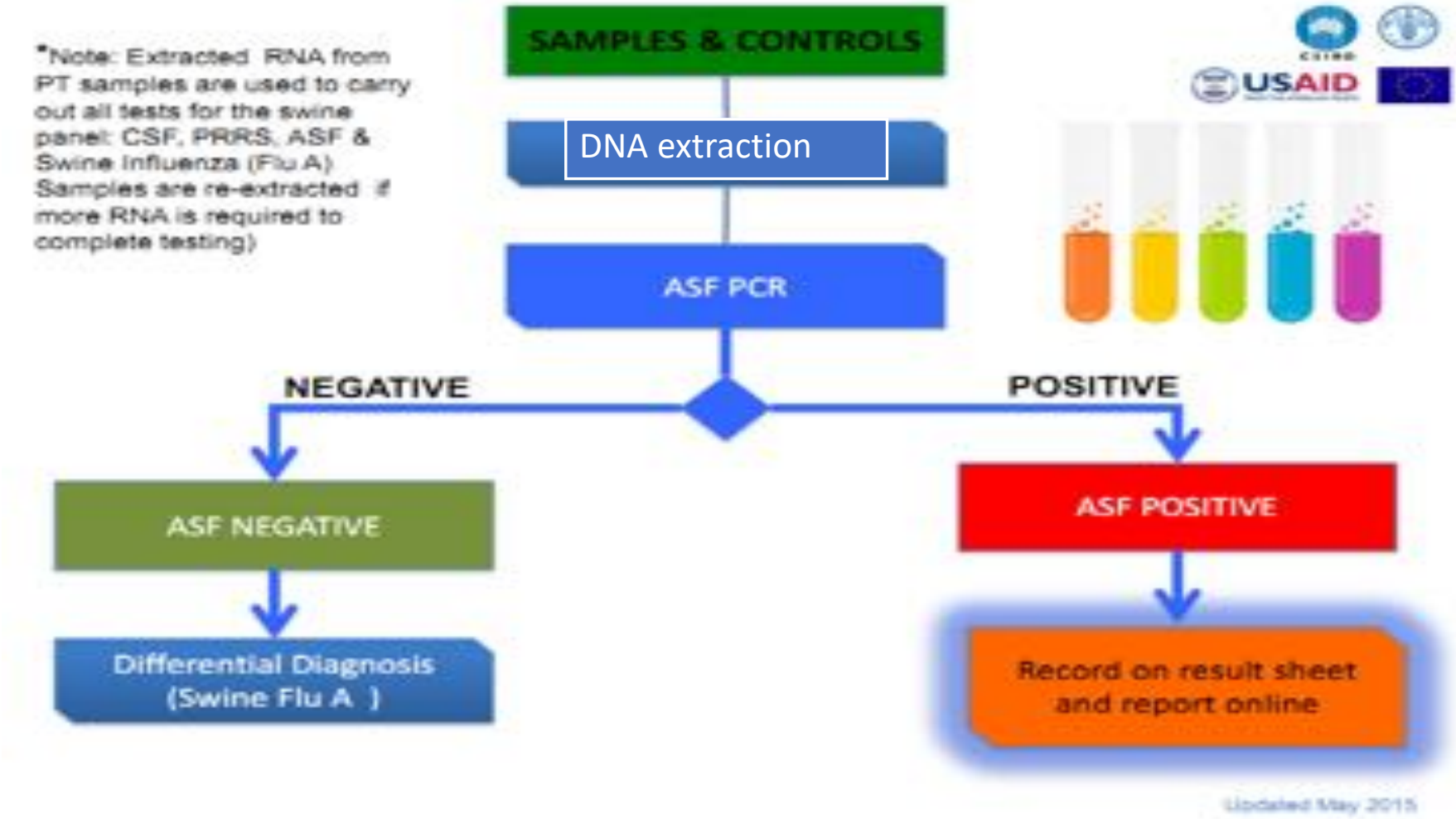
AUSTRALIAN ANIMAL HEALTH LABORATORY

GARA Research Alliance

CSIRO

FAO Emergency Regional Consultation on ASF Risk Reduction and Preparedness, 5th-7th September 2018, Bangkok

Australian Animal Health Laboratory



Portable qPCR and direct qPCR for ASF

Portable qPCR

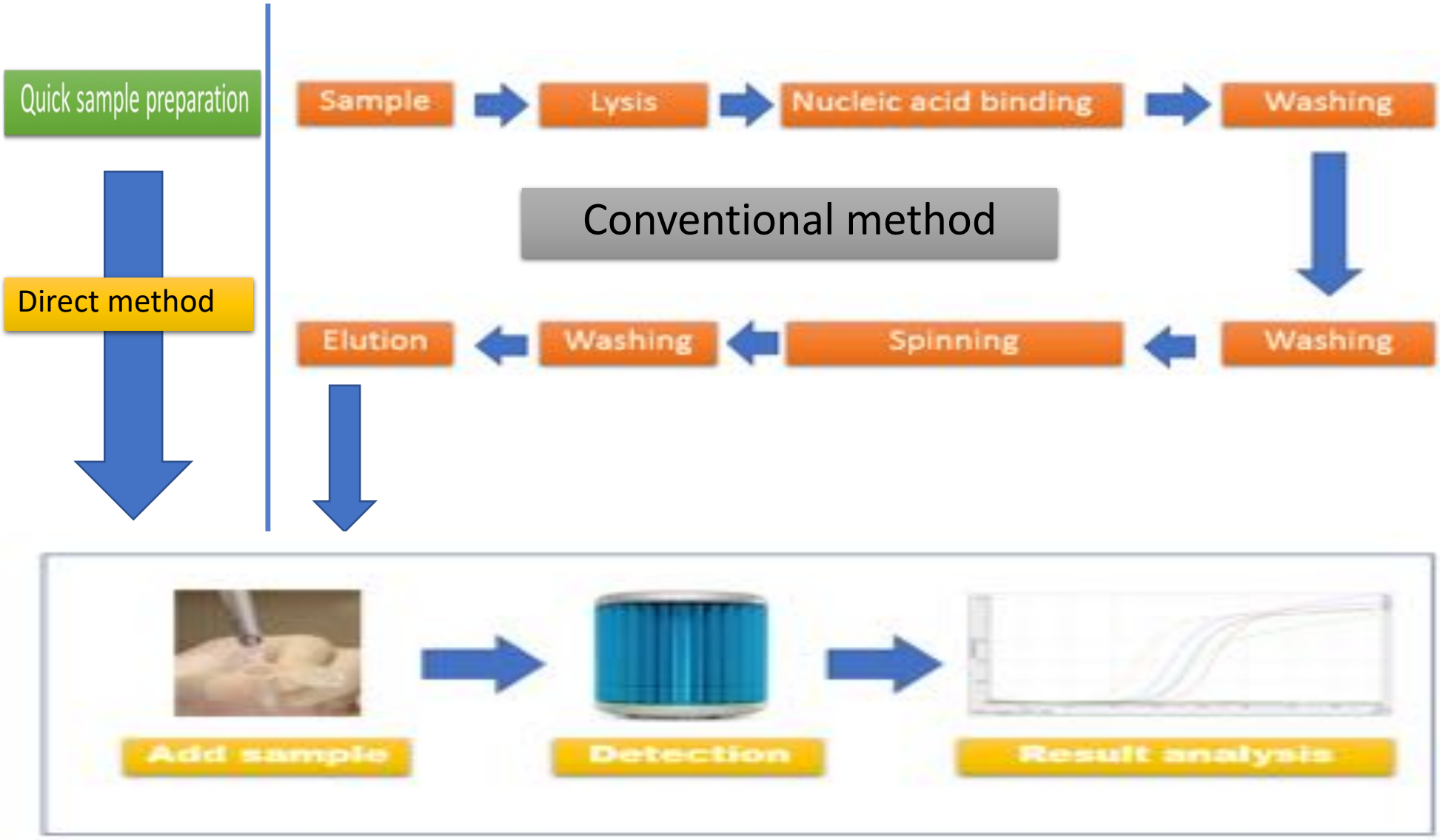
MYGO MINI

Portable qPCR

System Contain :

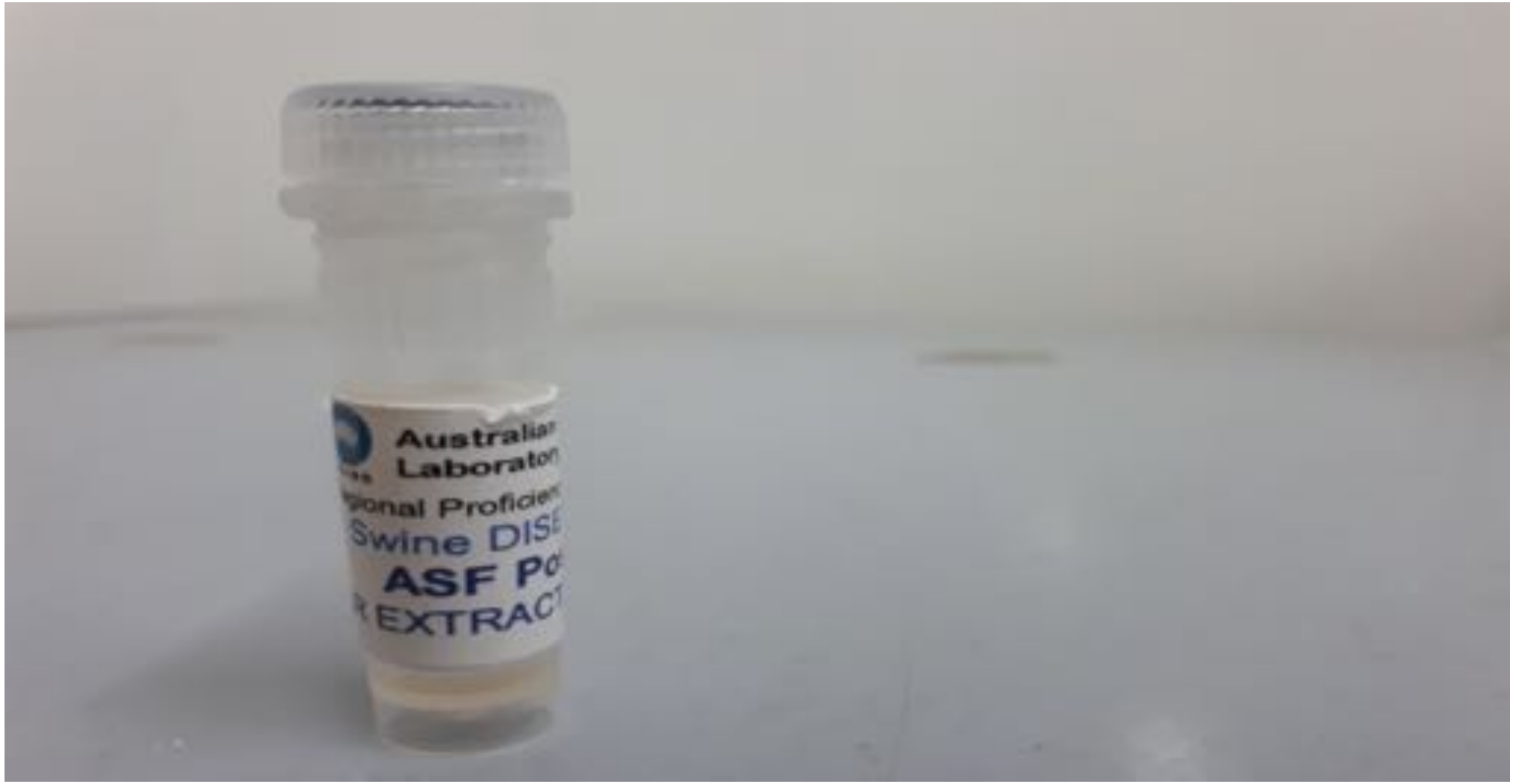
Real-Time PCR

Laptop	Power Generator	Adjustable Pipette	Hard Case Bag
			

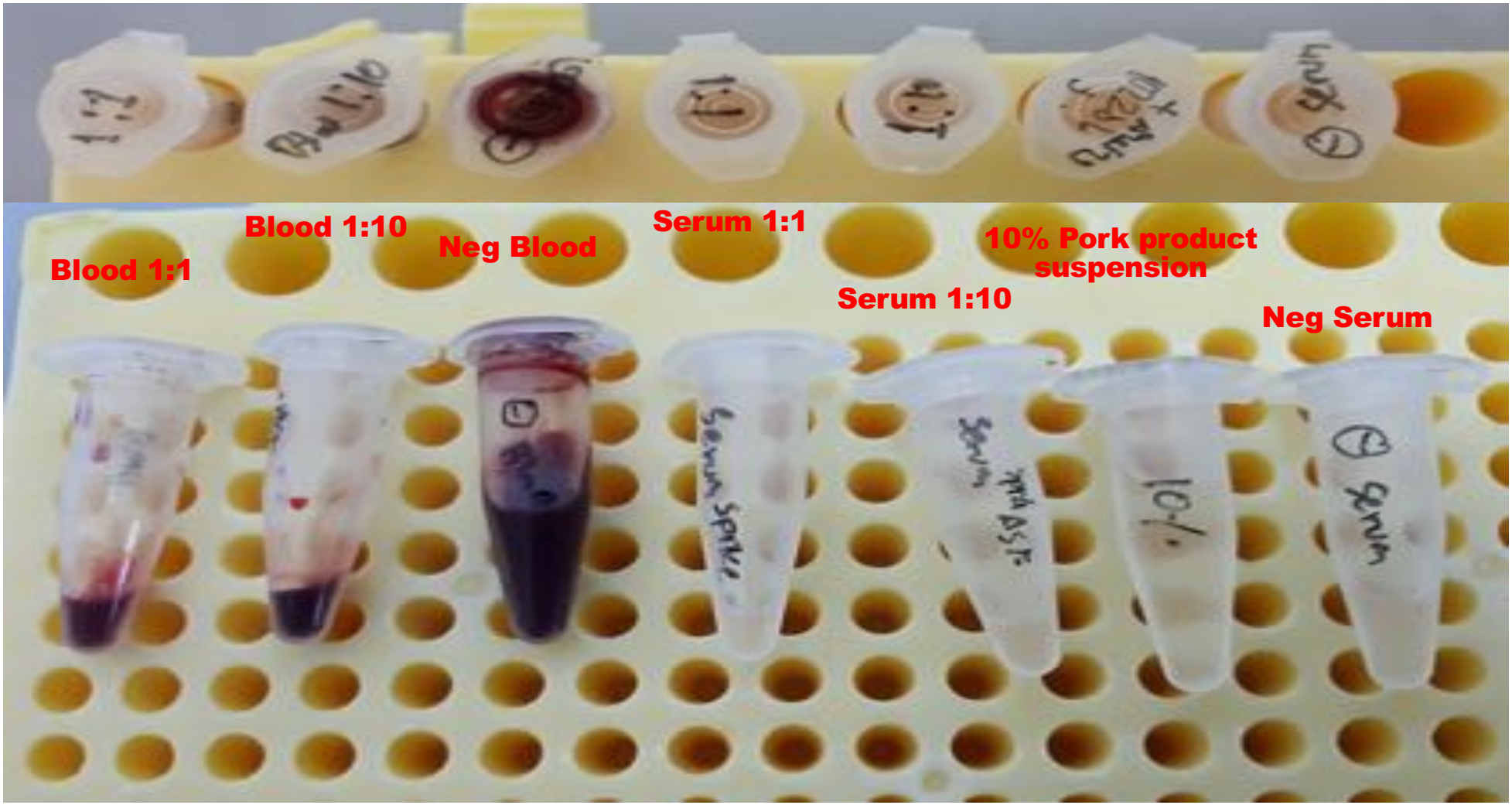


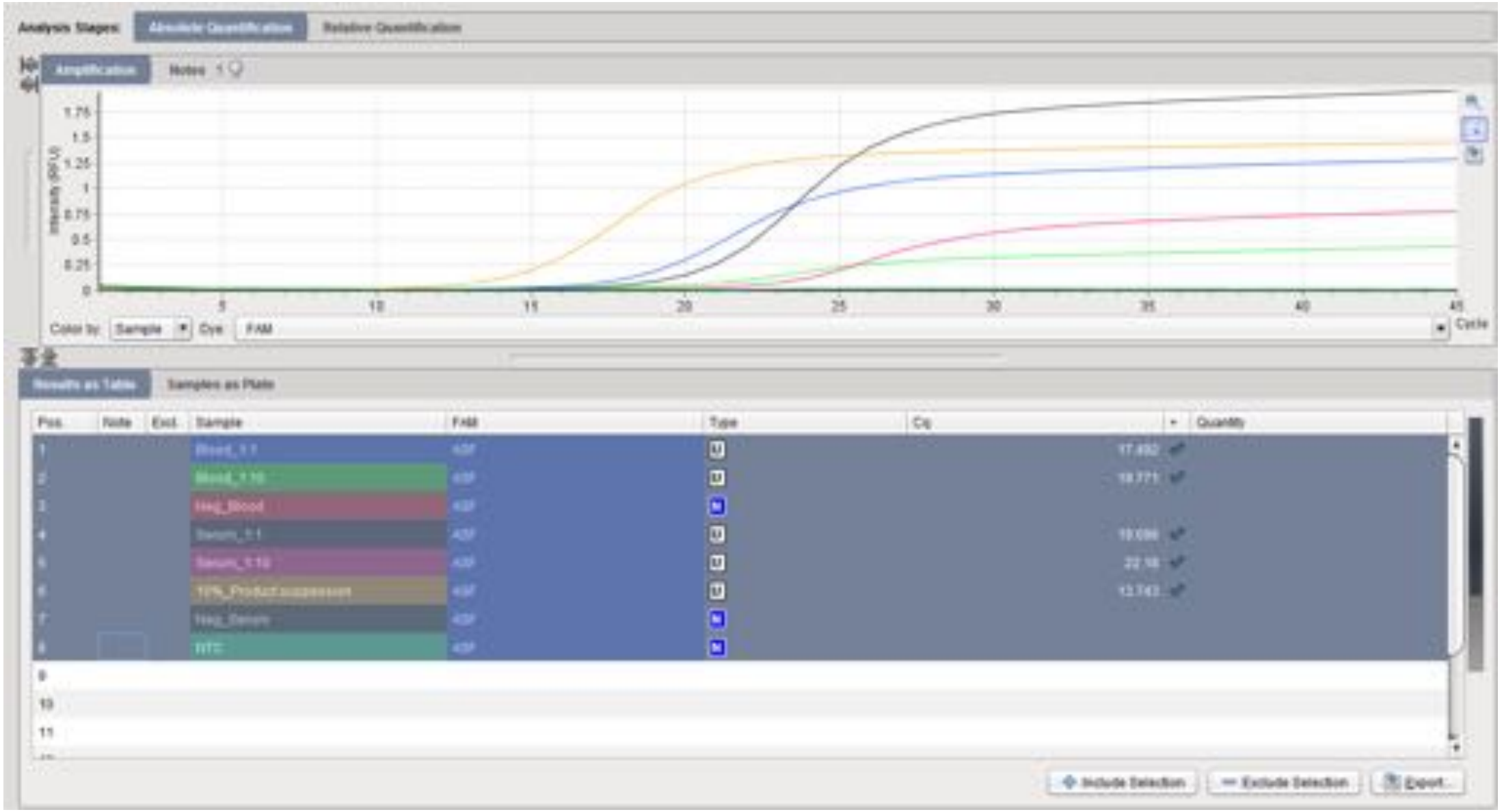
Modified from Coyote bioscience

Spiked Positive Control



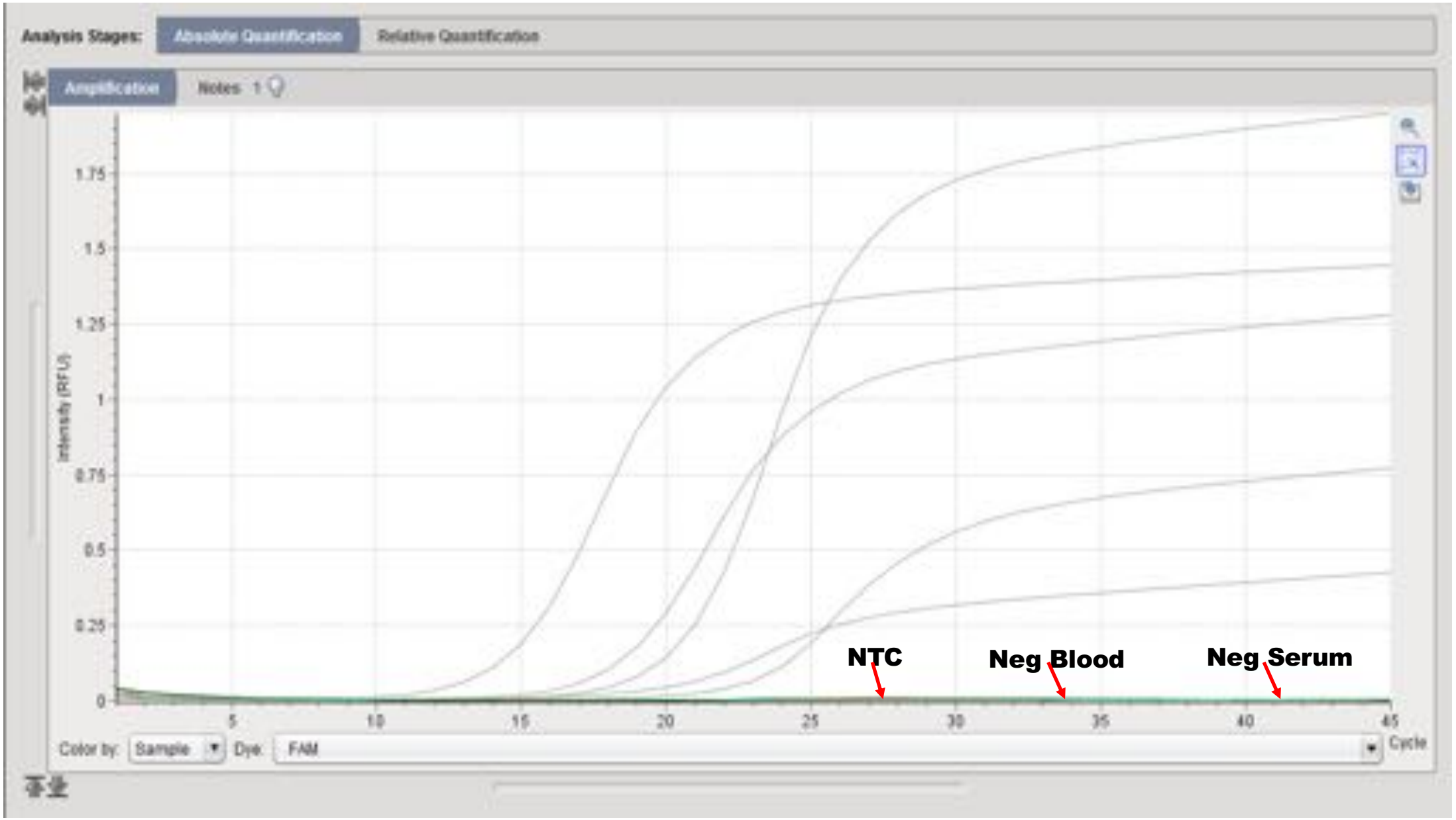
Direct qPCR ASF Kit

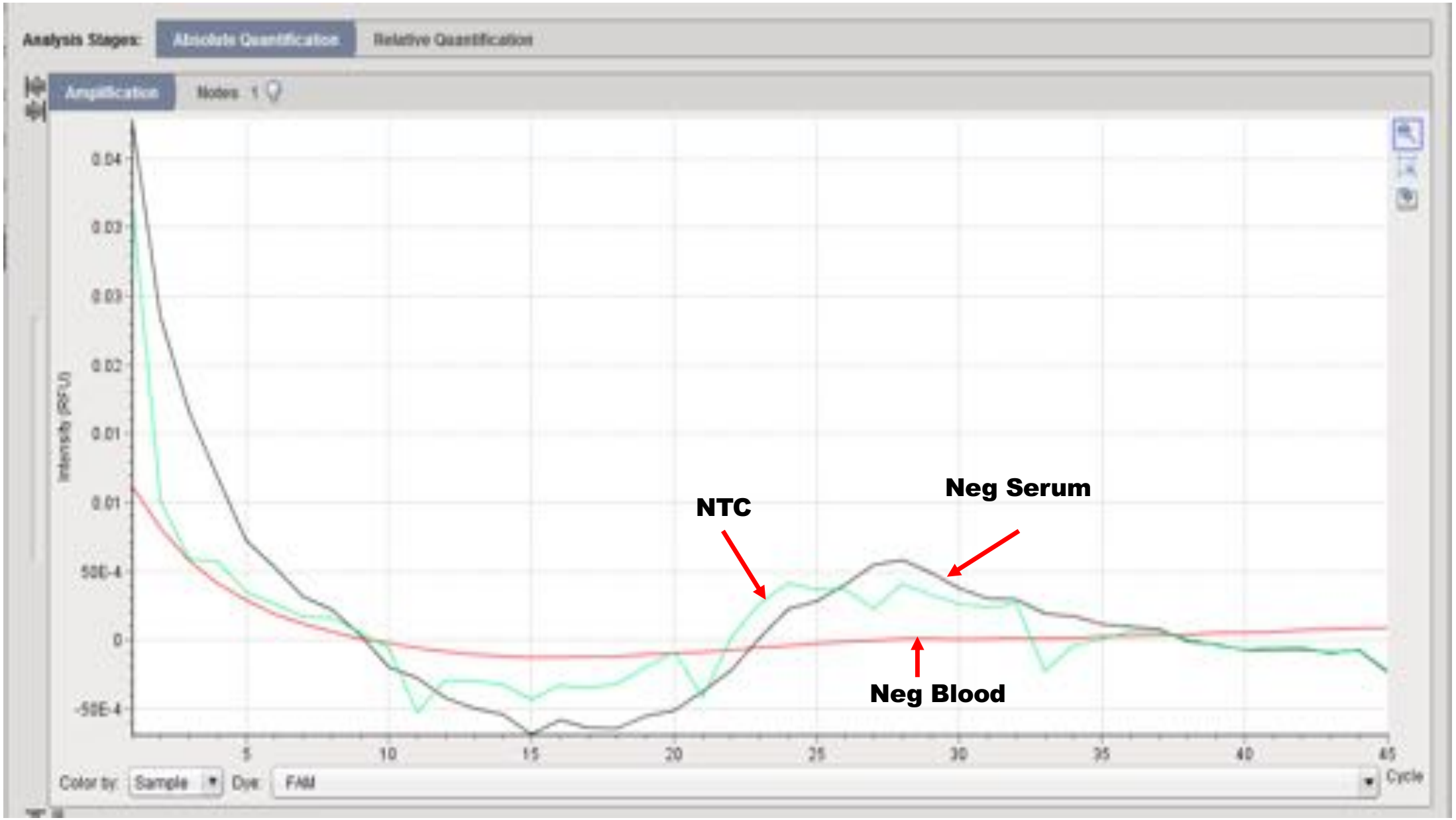



Analysis Stages:
Absolute Quantification
Relative Quantification
Amplification
Notes
Intensity (RFU)
Color by:
Sample
Cye
FAM
Cycle
Results as Table
Samples as Plate
Pos.
Note
Excl.
Sample
FAM
Type
Cq
Quantity
Include Selection
Exclude Selection
Export...

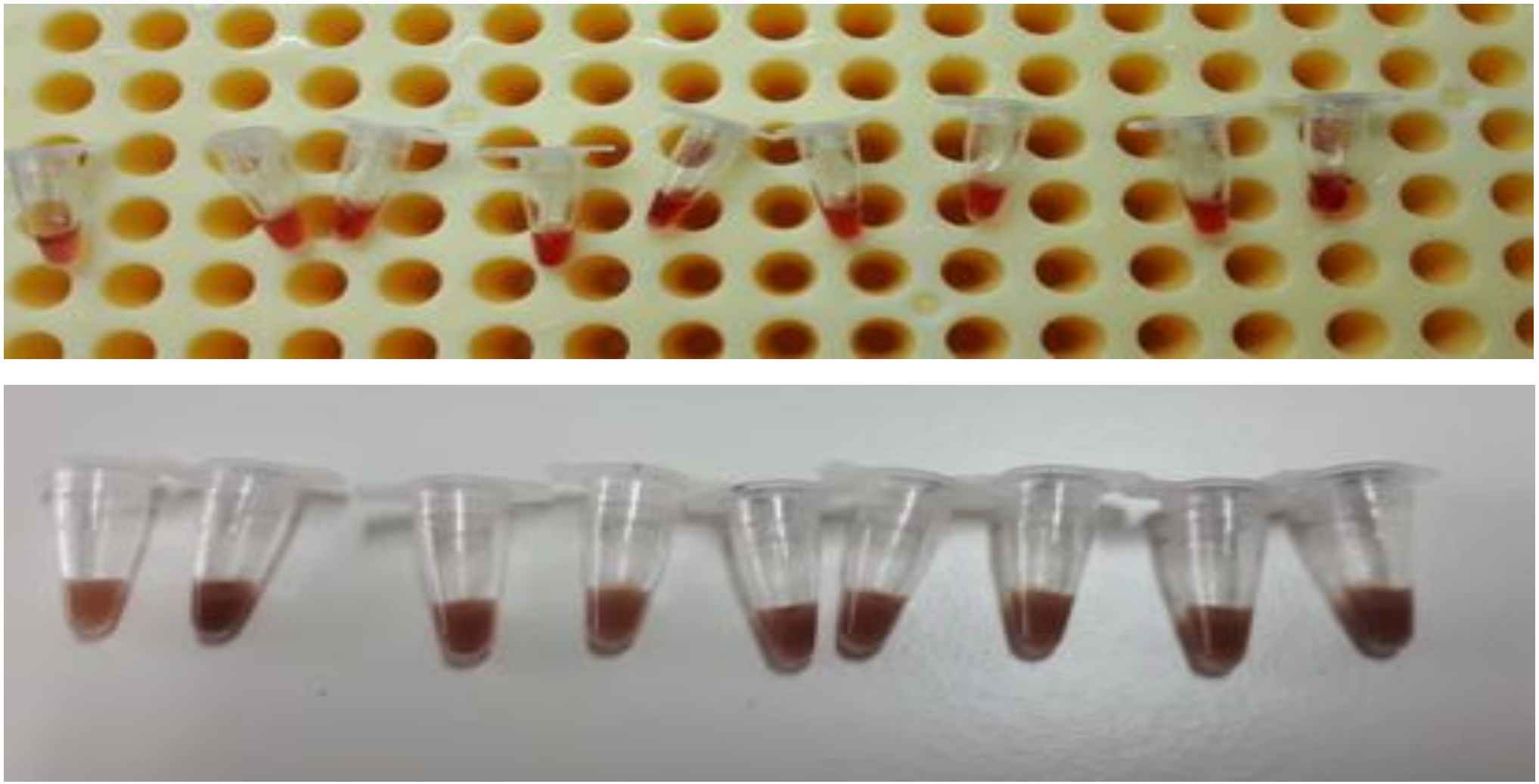

Serum 1:1
10% Pork product suspension
Blood 1:1
Serum 1:10
Blood 1:10


Analysis Stages:
Absolute Quantification
Relative Quantification
Amplification
Notes
1.25
1
0.75
0.5
0.25
0
Intensity (RFU)
5
10
15
20
25
30
35
40
45
Cycle
Color by:
Sample
Dye:
FAM
10% Pork product suspension
Blood 1:1
Serum 1:1
Serum 1:10
Blood 1:10

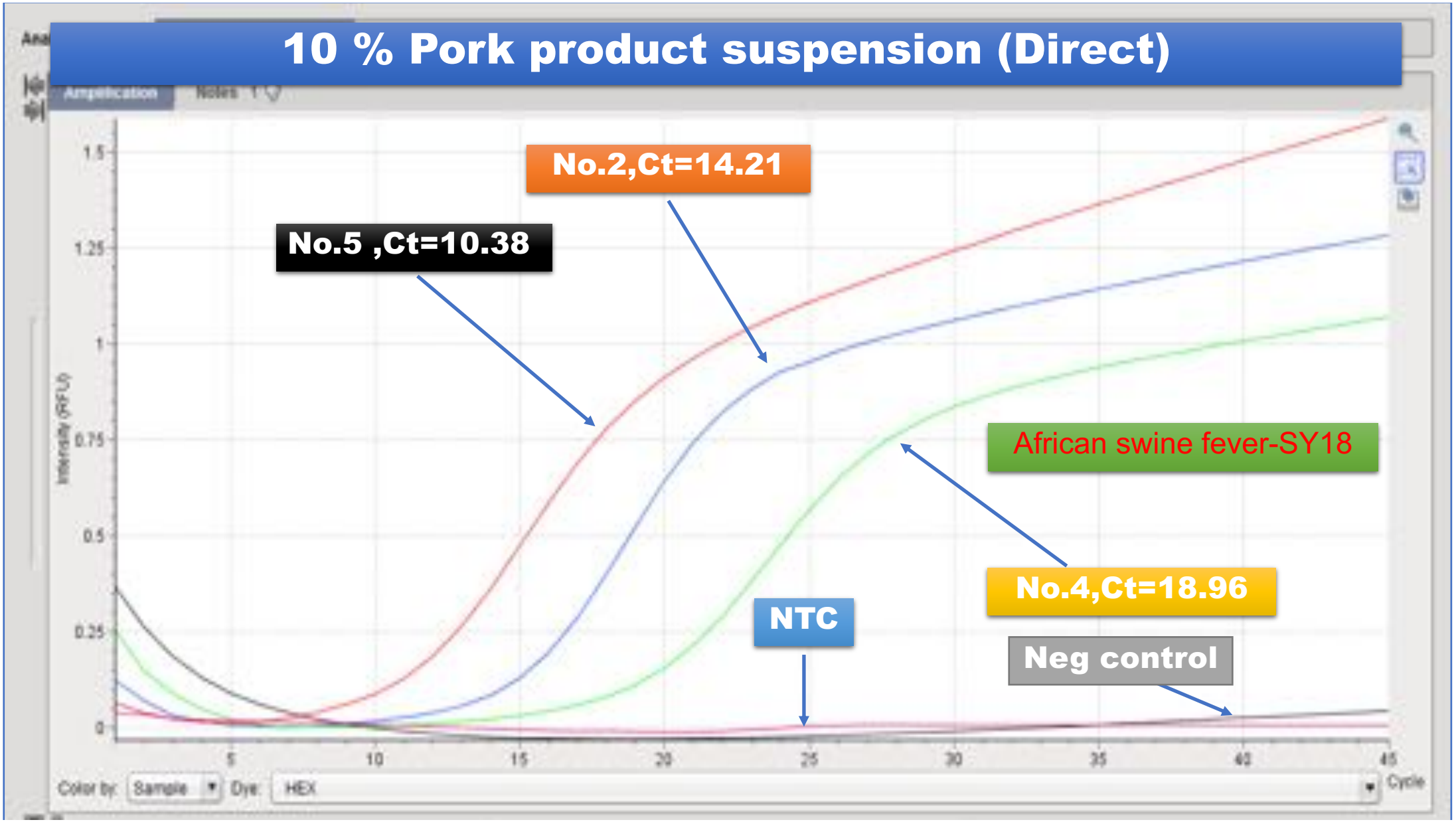

Analysis Stages:
Absolute Quantification
Relative Quantification
Amplification
Notes
Intensity (RFU)
1.75
1.5
1.25
1
0.75
0.5
0.25
0
5
10
15
20
25
30
35
40
45
Cycle
NTC
Neg Blood
Neg Serum
Color by:
Sample
Dye:
FAM


Analysis Stages:
Absolute Quantification
Relative Quantification
Amplification
Notes
0.04
0.03
0.03
0.02
0.01
0.01
50E-4
0
-50E-4
Intensity (RFU)
NTC
Neg Serum
Neg Blood
5
10
15
20
25
30
35
40
45
Cycle
Color by:
Sample
Dye:
FAM

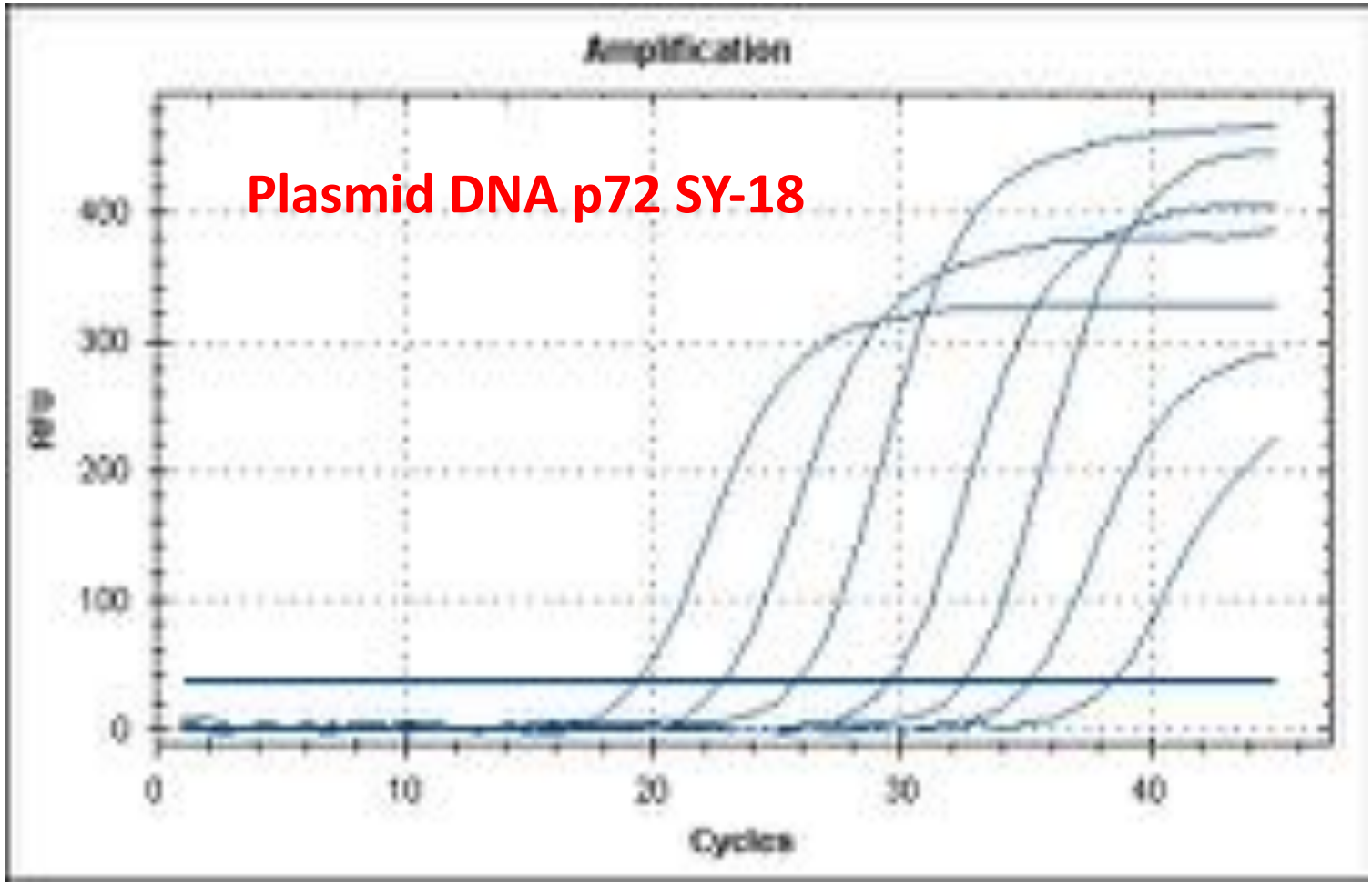
Before and After



10 % Pork product suspension (Direct)

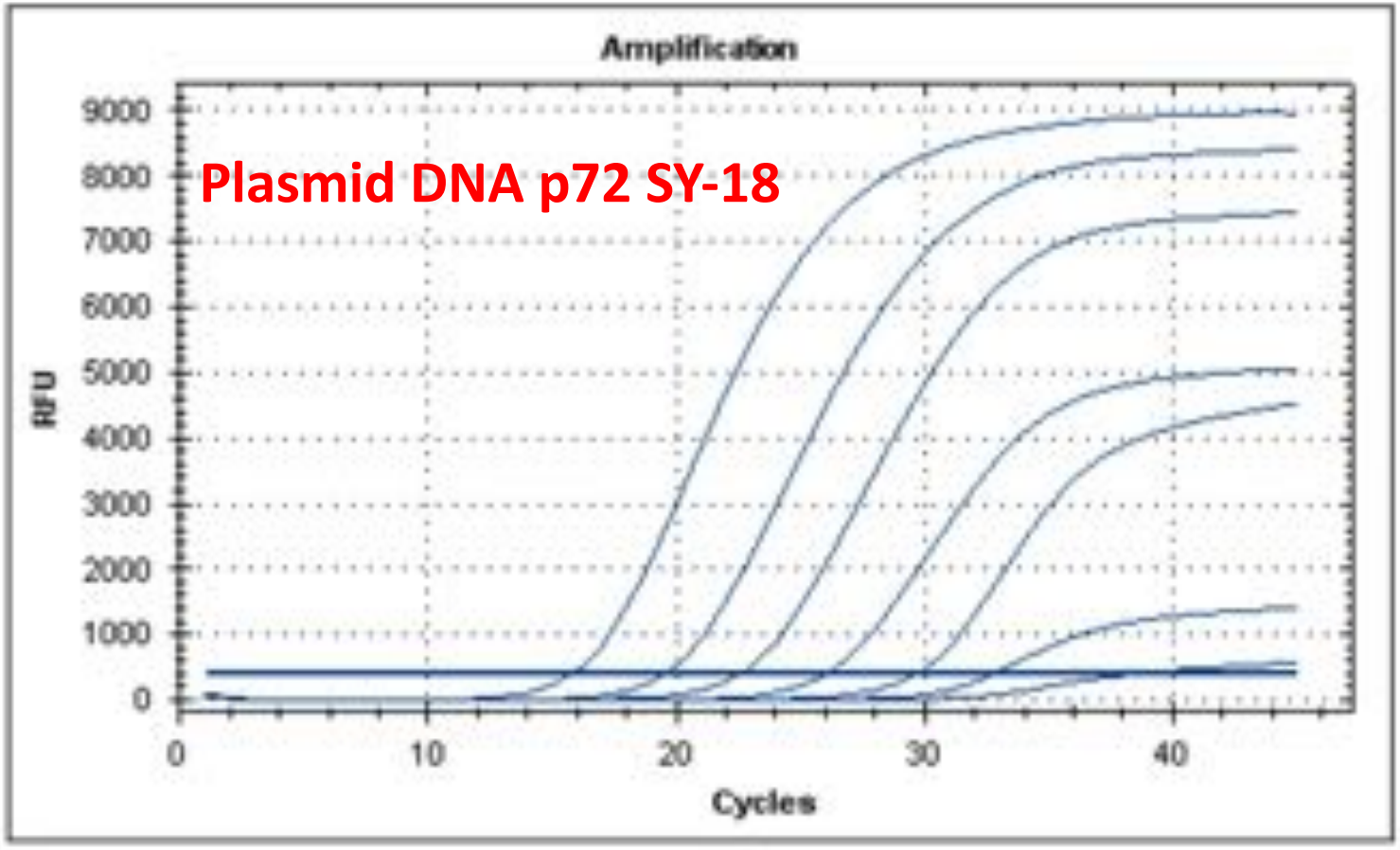


Spiked blood dilution



Dilution	Cq
-3	19.29
-4	22.74
-5	25.75
-6	29.48
-7	32.48
-8	35.19
-9	38.51

Spiked 10% organ suspension dilution



Samples	Cq
-3	15.41
-4	19.19
-5	22.18
-6	25.66
-7	29.28
-8	32.31
-9	36.84

Portable qPCR for Early Detection of ASF Virus

		Sampling	Sample Transportation	Testing	Total time
	Portable qPCR+Direct qPCR kit	1 Hr.		(Running time ~1.30) <2Hr.	3 Hr.<
	Lab-based qPCR	1 Hr.	Days	2Hr.	Days+3 Hr.

Modified from Ken inui and David williams


TROLL