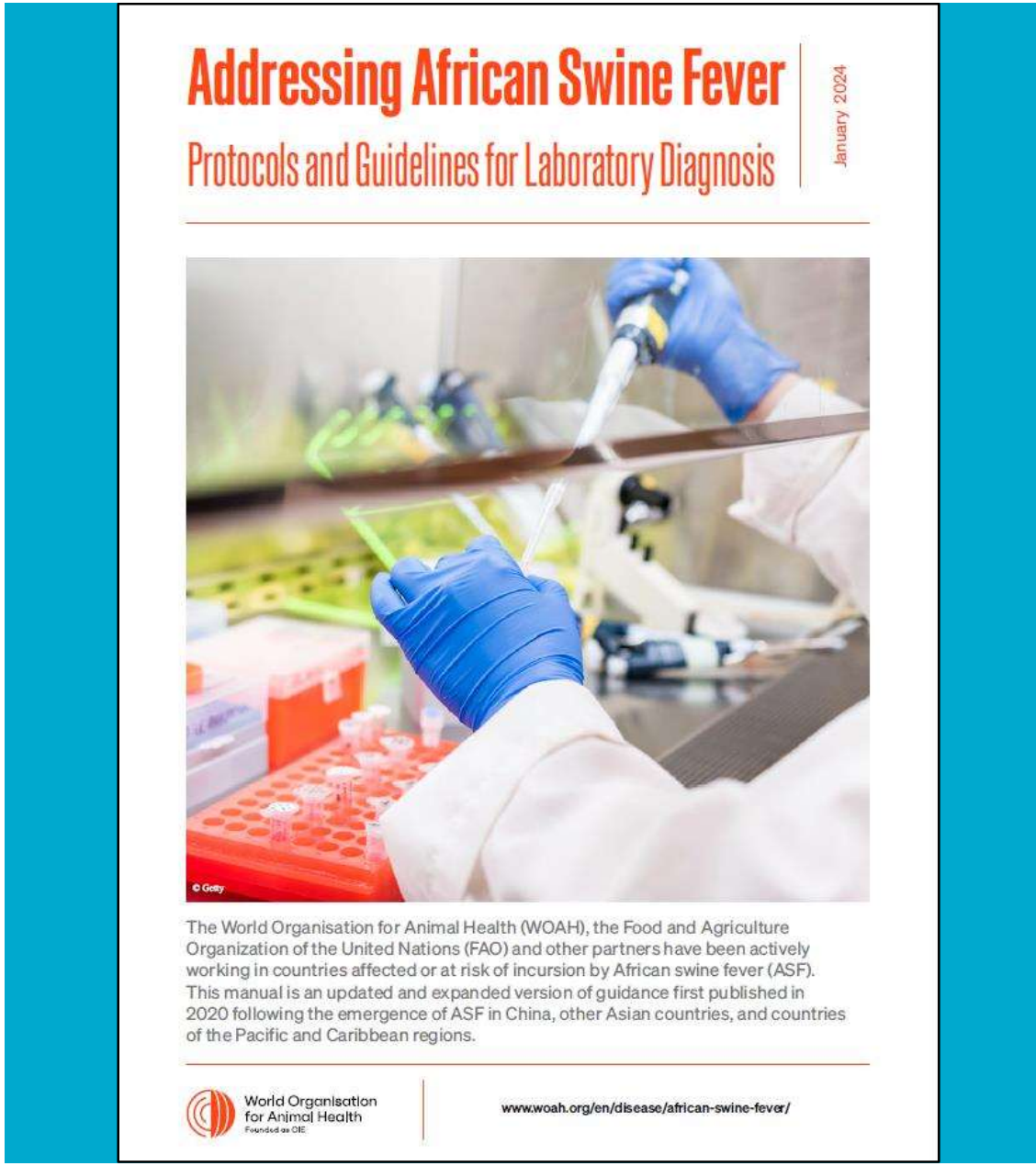
Addressing African Swine Fever: Protocols and Guidelines for Laboratory Diagnosis

Regional Seminar for WOAH National Focal Points for Veterinary Laboratories

David Williams | 17th July 2024



Protocols and Guidelines for Laboratory Diagnosis

- Addresses detection of:
  - Virulent and variant forms of ASFV
  - Authorised LAVs using DIVA PCRs
  - Technical procedures and guidance
  - Updates and extends previous version (2020)
- Developed in consultation with the WOAH ASF Reference Laboratory Network, based on WOAH recommendations for laboratory diagnosis of ASF
- *Available online*

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African Swine Fever Reference Laboratory Network

World Organisation for Animal Health
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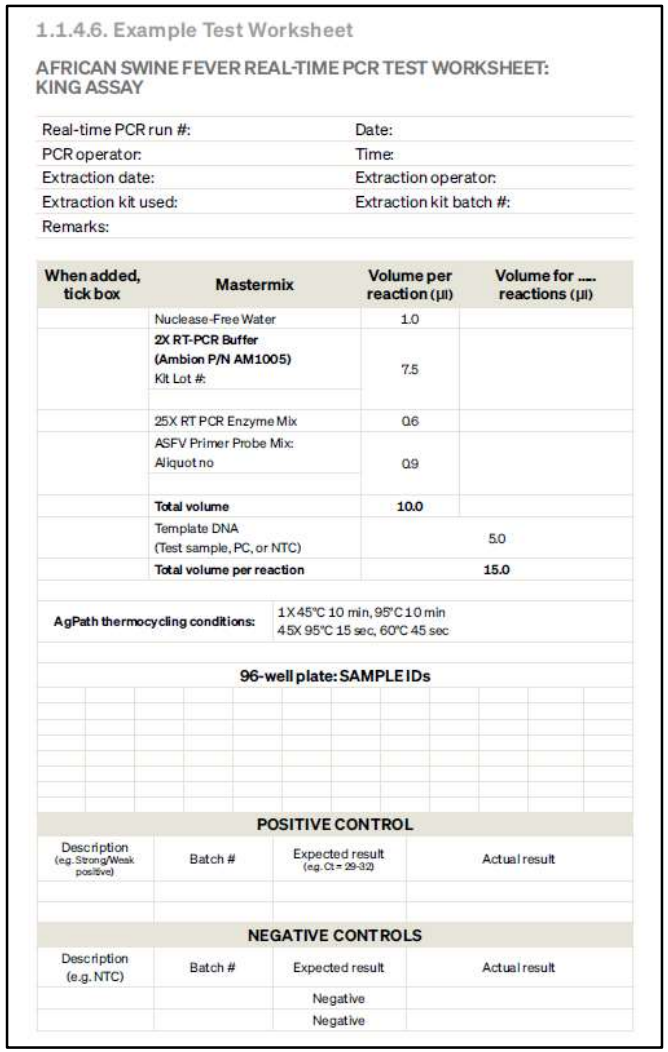
1. Molecular Diagnosis by PCR

- Overview of PCR testing and currently available tests
- For each assay:
  - Purpose
  - Suitable sample types (expanded in first protocol)
  - Extraction protocol from clinical specimens and pork products (expanded in first protocol)
  - PCR protocol
    - Kits and controls
    - Preparation of primer/probe mix and master mix
    - Programming PCR machine
    - Analysis and QC
  - Example Test Worksheet



1.1.4.6. Example Test Worksheet

AFRICAN SWINE FEVER REAL-TIME PCR TEST WORKSHEET: KING ASSAY

Real-time PCR run #:	Date:
PCR operator:	Time:
Extraction date:	Extraction operator:
Extraction kit used:	Extraction kit batch #:
Remarks:	

When added, tick box	Mastermix	Volume per reaction (µl)	Volume for ..... reactions (µl)
	Nuclease-Free Water	1.0	
	2X RT-PCR Buffer (Ambion P/N AM1005) Kit Lot #:	7.5	
	25X RT PCR Enzyme Mix	0.6	
	ASFV Primer Probe Mix: Aliquot no	0.9	
	Total volume	**10.0**	
	Template DNA (Test sample, PC, or NTC)		5.0
	Total volume per reaction		**15.0**

AgPath thermocycling conditions: 1X 45°C 10 min, 95°C 10 min; 45X 95°C 15 sec, 60°C 45 sec

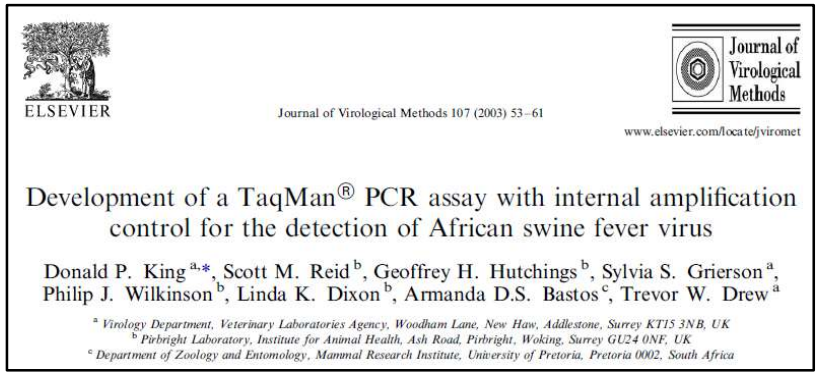
96-well plate: SAMPLE IDs

POSITIVE CONTROL			
Description (eg. Strong/Weak positive)	Batch #	Expected result (eg. Ct = 29-32)	Actual result

NEGATIVE CONTROLS			
Description (e.g. NTC)	Batch #	Expected result	Actual result
		Negative	
		Negative	

1.1 PCR Protocol: King assay

- WOAH-recommended, frontline test targeting the *B646L* gene (p72)
- *Included in previous version of manual, and kits provided to various veterinary laboratories in SE Asia by FAO and WOAH in recent years*
- Used to **detect all ASFV genotypes**, including pandemic genotype II and genotype I/II recombinants reported in China (Zhao et al., 2022)
- **Validated** using European and African ASFV isolates belonging to different genotypes, including genotype II (King et al., 2003; Gallardo et al., 2015)



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]

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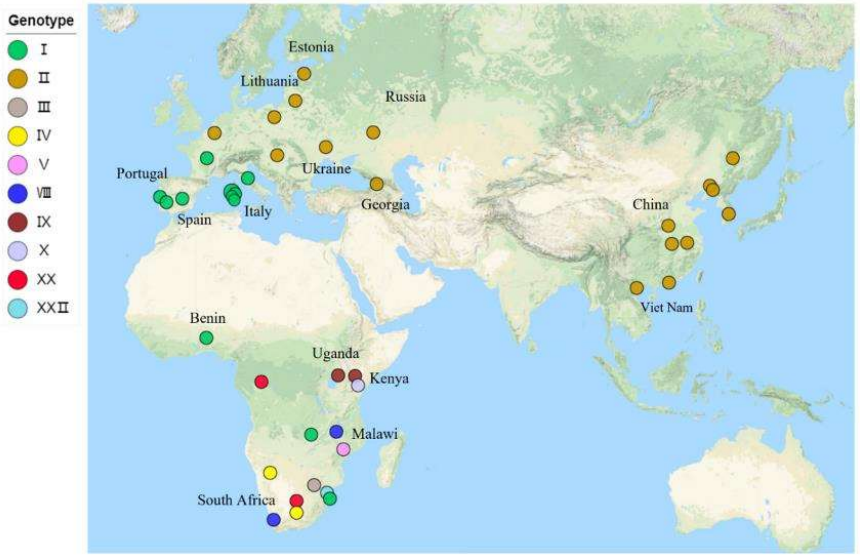
[b] Pirbright Laboratory, Institute for Animal Health, Ash Road, Pirbright, Woking, Surrey GU24 0NF, UK
[c] Department of Zoology and Entomology, Mammal Research Institute, University of Pretoria, Pretoria 0002, South Africa



Shen, et al. *Viruses* **2022**, *14*, 889.

PCR detection of ASFV variants

- **Naturally occurring** lower virulence isolates of genotype II ASFV reported in China since 2020
- Encode different combinations of point mutations, insertions, and deletions in various genes
  - *E.g. deletions in* EP402R *(CD2v), and multigene family (*MGF-*)* 360 *and* 505 *genes*
- To **differentiate genotype II variants from wildtype virus**, multiplex assay targeting *B646L*, *MGF-360* and *EP402R* genes can be used

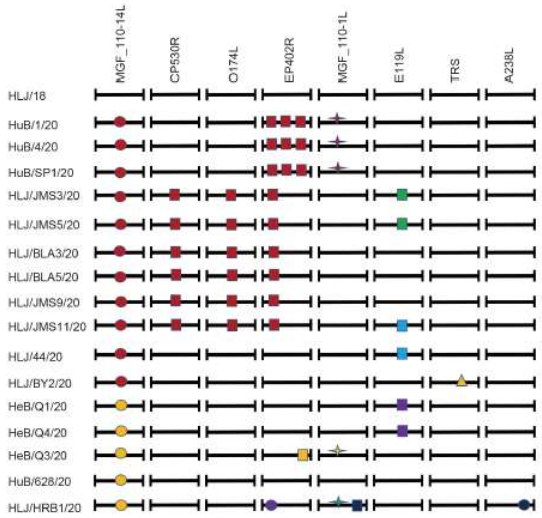
Identification of a natural variant of African swine fever virus in China

ZHANG Yanyan, ZHANG Jingyuan, YANG Jinjin, YANG Jinmei, HAN Xun, MI Lijuan, ZHANG Fei, QI Yu, ZHANG Shoufeng, WANG Ying, ZHOU Xintao, YUE Huixian, WANG Shuchao, CHEN Teng*, HU Rongliang* (*Military Veterinary Institute, Academy of Military Medical Science*, PLA *Academy of Military Science, Changchun* 130122, *China*)

Emergence and prevalence of naturally occurring lower virulent African swine fever viruses in domestic pigs in China in 2020

Encheng Sun[1†], Zhenjiang Zhang[1†], Zilong Wang[1†], Xijun He[1†], Xianfeng Zhang[1], Lulu Wang[1], Wenqing Wang[1], Lianyu Huang[1], Fei Xi[1], Haoyue Huangfu[1], Ghebremedhin Tsegay[1], Hong Huo[1], Jianhong Sun[1], Zhijun Tian[1], Wei Xia[1], Xuewu Yu[2], Fang Li[1], Renqiang Liu[1], Yuntao Guan[1], Dongming Zhao[1*] & Zhigao Bu[1*]

[1]*State Key Laboratory of Veterinary Biotechnology, National High Containment Facilities for Animal Diseases Control and Prevention, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin 150069, China;*
[2]*College of Animal Science and Technology, Inner Mongolia University for Nationalities, Tongliao 028000, China*



1.2 PCR Protocol: CAHEC Triplex assay

Interpretation	Test results		
	P72 (*B646L*)	**CD2v** *(EP402R)*	**MGF**
ASFV **pandemic strain** positive	+	+	+
ASFV **CD2v gene deletion** strain positive	+	-	+
ASFV **MGF-360-14L gene deletion** strain positive	+	+	-
ASFV **CD2v and MGF gene double deletion** strain positive	+	-	-
ASFV negative	-	-	-

1.3. PCR Protocol: ASFV *I177L* Assay
1.4. PCR Protocol: ASFV *MGF360-12L* Assay

- Two USDA genotype II LAVs approved for use in Vietnam
  - ASFV-ΔI177L (Navetco)
  - ASFV-ΔMGF (AVAC)
- Accompanying DIVA PCR assays reported by USDA (Velazquez-Salinas et al., 2021)
- Samples that test positive for p72 and negative for *I177L* or *MGF360-12L* indicate presence of ASFV-ΔI177L or ASFV-ΔMGF LAVs, respectively

Vietnam approves commercial use of first African swine fever vaccines

By **Reuters**

July 25, 2023 2:53 AM GMT+11 · Updated 9 months ago

Pigs are seen at a farm outside Hanoi, Vietnam September 20, 2019. Picture taken September 20, 2019. REUTERS/Kham/File Photo

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Development Real-Time PCR Assays to Genetically Differentiate Vaccinated Pigs From Infected Pigs With the Eurasian Strain of African Swine Fever Virus

Lauro Velazquez-Salinas[1,2], Elizabeth Ramirez-Medina[1], Ayushi Rai[1,3], Sarah Pruitt[1], Elizabeth A. Vuono[1,4], Nallely Espinoza[1], Douglas P. Gladue[1*] and Manuel V. Borca[1*]*

[1] Agricultural Research Service, United States Department of Agriculture, Plum Island Animal Disease Center, Greenport, NY,

1.5. PCR Protocol: Genotype I Assay

- Emergence of **low virulent genotype I ASFV** reported in China, associated with chronic ASF
  - Genome resembles early attenuated European strains (NH/P68 and OURT88/3)
  - Contains particular pattern of deletions in *MGF* and *EP402R* genes
- Assay developed by ACDP for specific detection of genotype I isolates (targets *MGF505-3R* gene)

Emerging Microbes & Infections
2021, VOL 10
https://doi.org/10.1080/22221751.2021.1999779

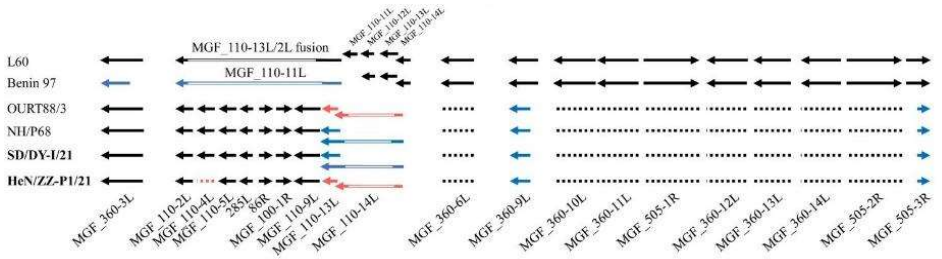
ORIGINAL ARTICLE

OPEN ACCESS

Genotype I African swine fever viruses emerged in domestic pigs in China and caused chronic infection

Encheng Sun*, Lianyu Huang*, Xianfeng Zhang*, Jiwen Zhang*, Dongdong Shen*, Zhenjiang Zhang, Zilong Wang, Hong Huo, Wenqing Wang, Haoyue Huangfu, Wan Wang, Fang Li, Renqiang Liu, Jianhong Sun, Zhijun Tian, Wei Xia, Yuntao Guan, Xijun He, Yuanmao Zhu, Dongming Zhao and Zhigao Bu

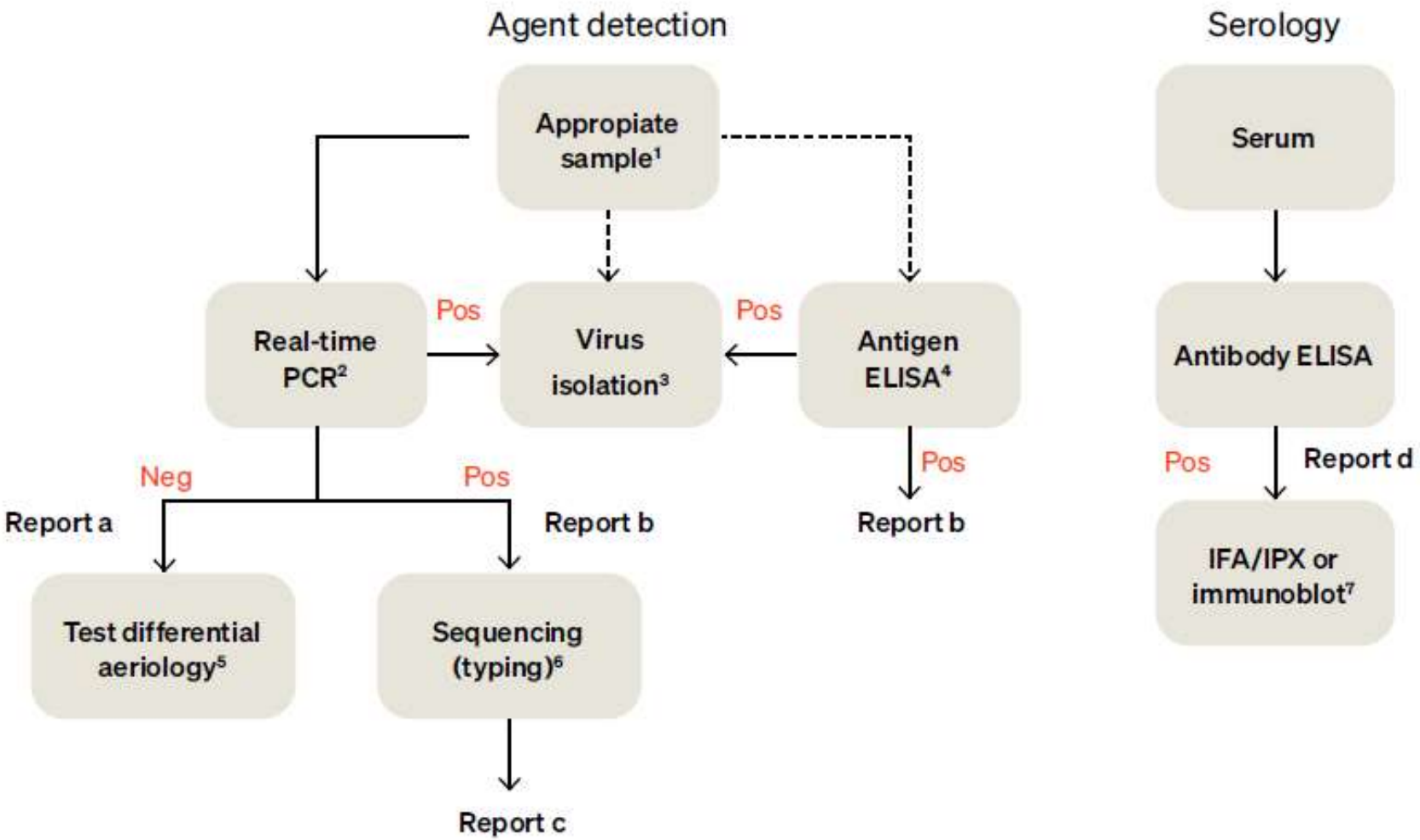
State Key Laboratory of Veterinary Biotechnology, National High Containment Facilities for Animal Diseases Control and Prevention, National African Swine Fever Para-reference Laboratory, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, People's Republic of China



2. Serology

- Infection of pigs with lower virulence ASFV variants associated with non-lethal, subacute and chronic disease → **seroconversion**
- The use of serological tests for laboratory diagnosis has therefore become important in regions where these viruses are circulating
- *Pivot from previous recommendations that emphasised the importance of PCR as the frontline detection method for China and countries in Southeast Asia and the Indo-Pacific, where the acute form of ASF first appeared*
- Several commercial and in-house serological test options available for various scenarios
  - Alternative sample types to serum can also be used

3. Algorithm for the Detection of ASF virus



4. Diagnostic Approaches for Different Scenarios

Purpose	ASF Status	Testing Method						
		Virus detection and identification					Antibody detection	
		ASFV generic PCR	Antigen ELISA*	ASFV variant/ DIVA PCR†	Sequencing/ typing	Virus isolation/ HAD	Antibody ELISA^	IPX/IFA/ IB
Outbreak investigation	Endemic	X	X	X	X	X	X	X
	Free	X	X		X	X	X	X
Active surveillance	Endemic	X	X	X			X	
	Free	X	X				X	X
Passive surveillance	Endemic	X	X	X			X	
	Free	X	X				X	X
Quarantine	Endemic						X#	
		X#	X#				X#	X

When ASFV is detected in a country previously free of ASF, samples should be submitted to a WOAH ASF Reference Laboratory for confirmatory testing and characterisation of the virus.

Australian Centre for Disease Preparedness

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