



Updates and Challenges on ASF Diagnostics

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WOAH Reference Laboratory for African swine fever
at the Australian Centre for Disease Preparedness

11th Standing Group of Experts on African Swine Fever
(SGE ASF) for Asia and the Pacific, Seoul, Republic of
Korea, 11th-13th August 2026

**African Swine Fever
Reference Laboratory Network**



World Organisation
for Animal Health
Founded as OIE

Australia's National Science Agency



Image courtesy of Dr. Myra Hosmillo, University of Cambridge

- Lab facilities
 - BSL2 = 2600 m²
 - BSL3 = 2900 m²
 - BSL4 = 400 m²
- Animal facilities
 - BSL3 = 955 m²
 - BSL4 = 127 m²
- Insectary
 - BSL3 = 250 m²

Australian Centre for Disease Preparedness (ACDP) is Australia's national biocontainment facility for laboratory diagnostics (ISO 17025) and research on terrestrial and aquatic animal disease threats, and zoonoses





ASF laboratory diagnosis at ACDP

- Multidisciplinary capability for laboratory diagnosis of ASF
- Diagnostic or confirmatory testing and virus characterisation for outbreak response and surveillance
 - Regional member countries
 - Disease exclusions for Australian domestic pigs, feral pig surveillance
- Diagnostic capacity building for regional animal health labs
 - Training and advice (diagnostics, biosafety)
 - Provide diagnostic reagents (PCR kits, pos controls)
 - Proficiency testing (PT): ISO/IEC 17043-accredited, regional (Asia-Pacific) programme for swine diseases, including ASF and CSF viruses



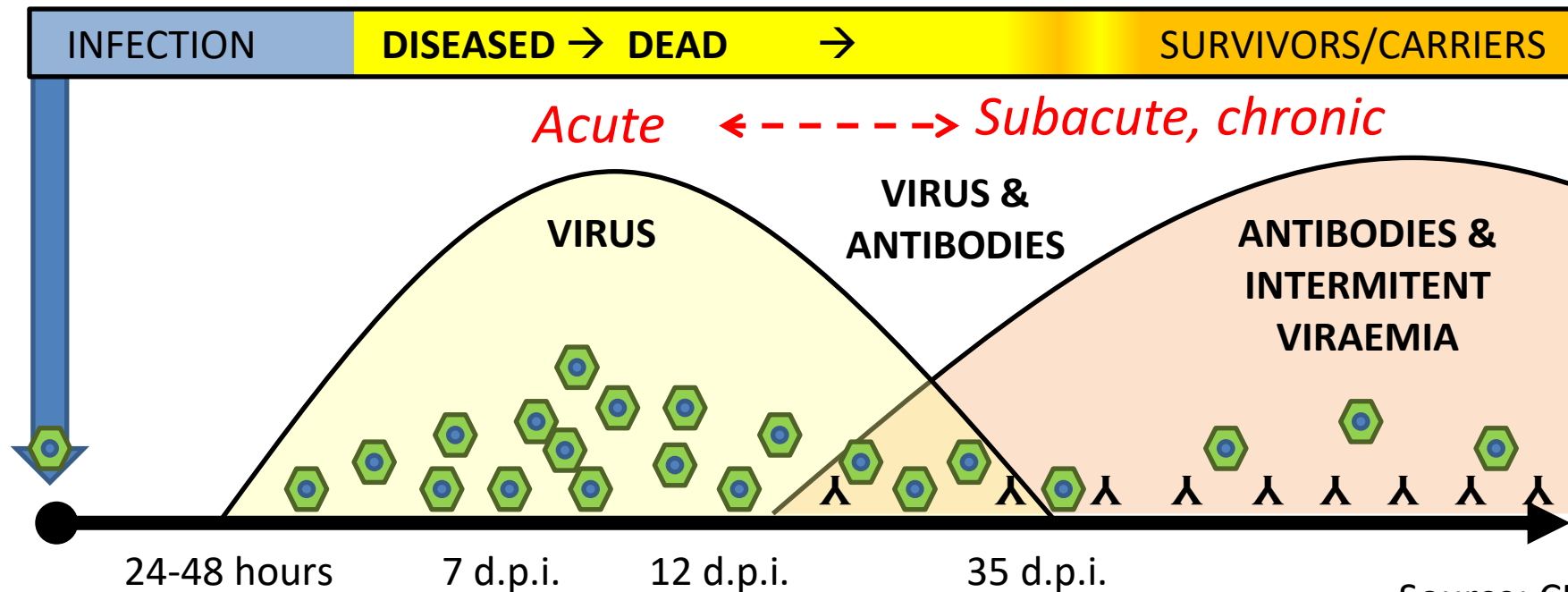
Laboratory Diagnostics for ASF

- ASF **cannot** be diagnosed based on clinical signs alone because of its similarity with other haemorrhagic diseases
- Laboratory testing is therefore essential for diagnosis and surveillance
- Laboratory techniques currently used enable confident diagnosis of ASF across a range of epidemiological situations



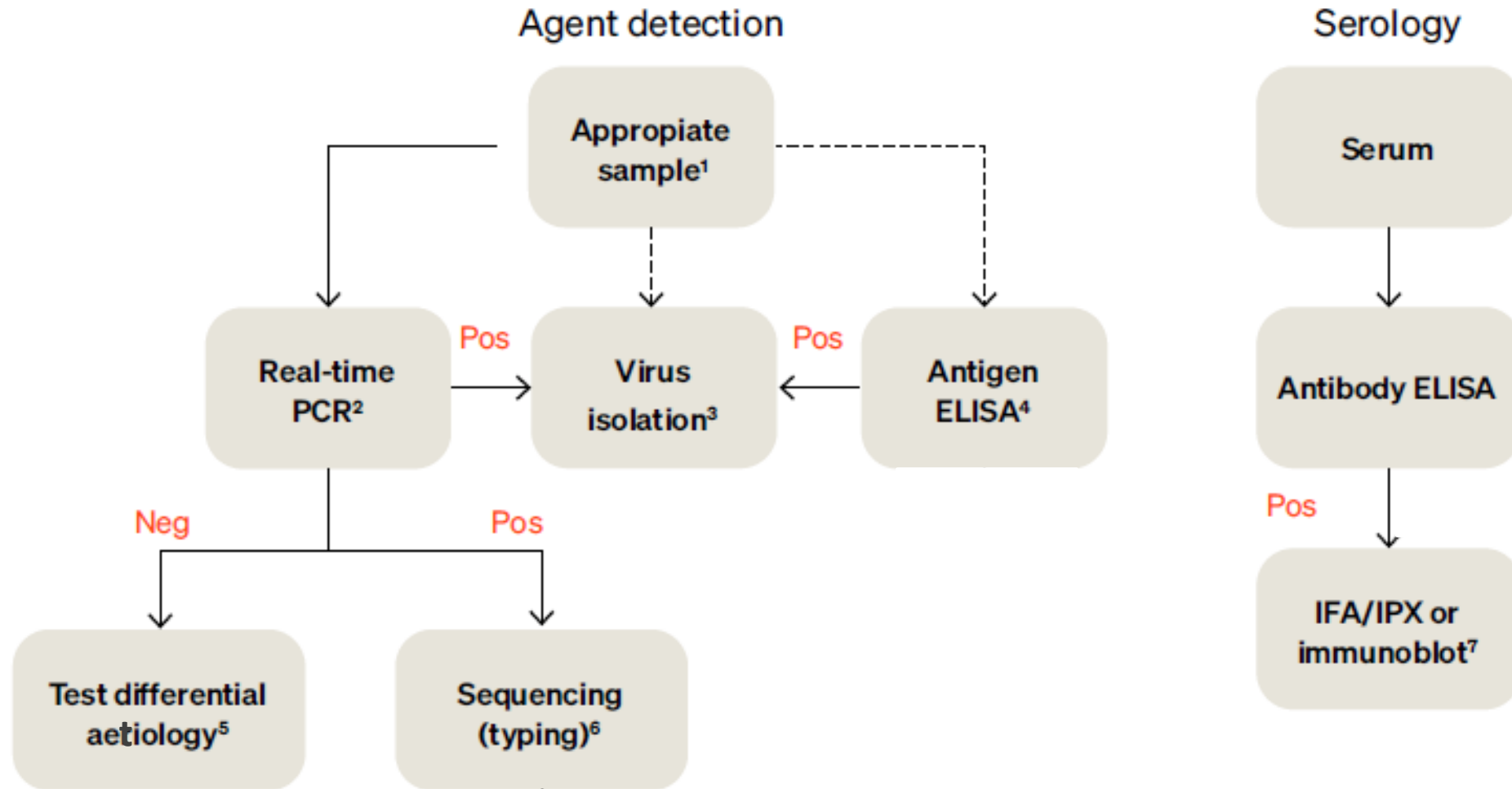
Diagnostics for ASF

- Acute disease: **virus detection (DNA, antigen)** most useful, serology of lower diagnostic value since most pigs die before antibody response (7-10d)
- Chronic or subacute disease: both **virus detection** and **serology** can be used since pigs typically survive long enough to seroconvert



Source: CISA-INIA and UCM

Laboratory diagnostic algorithm for ASF



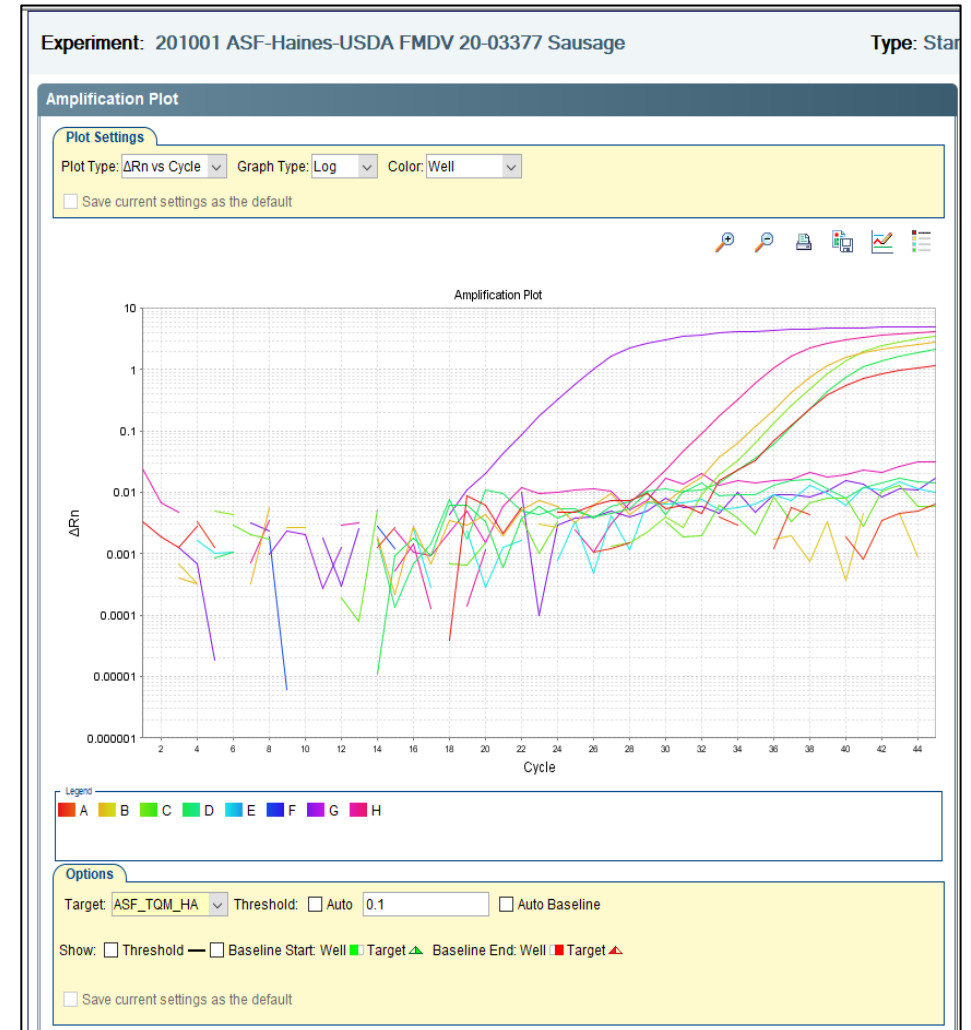
Specimens for ASF Laboratory Diagnosis

- **Recommended** specimens:
 - Whole blood (serum, plasma), organs (especially spleen, lymph nodes, lung, tonsils, kidneys)
- **Alternative** specimens:
 - Swabs: nasal, oral, faecal/faeces, fluid from the peritoneal cavity
 - Bone marrow (e.g. dead animals – best preserved tissue)
 - Pen-based: oral fluids, faeces
 - Meat juice exudate (virus and antibody)
 - Superficial inguinal lymph nodes – convenient and low contamination risk
- **Pork** specimens:
 - Fresh, (un)cooked meat, dried (biltong, jerky, sausages etc)
- Consider **swab sampling systems** (wet/dry) to preserve sample if cold chain problematic (e.g. remote locations)



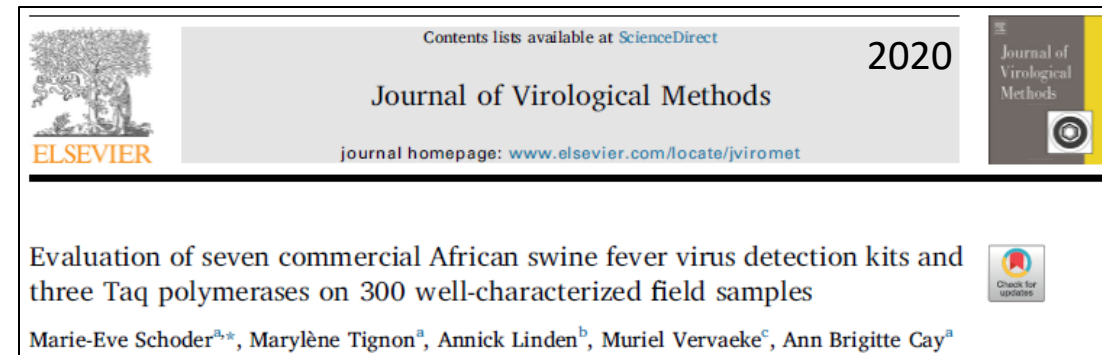
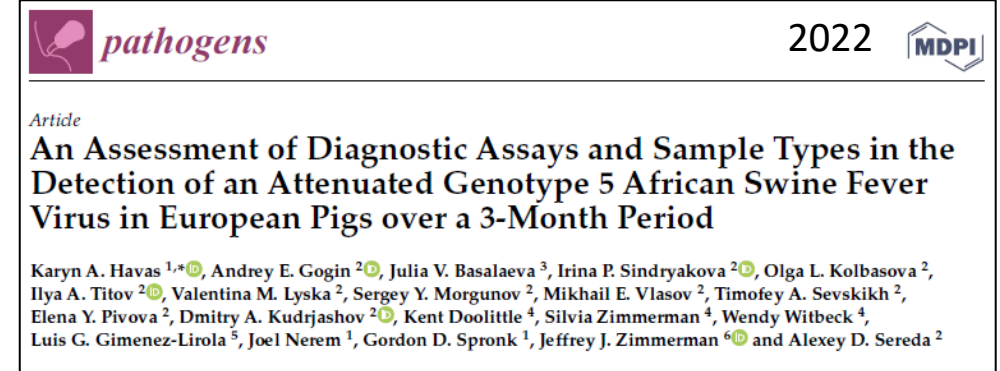
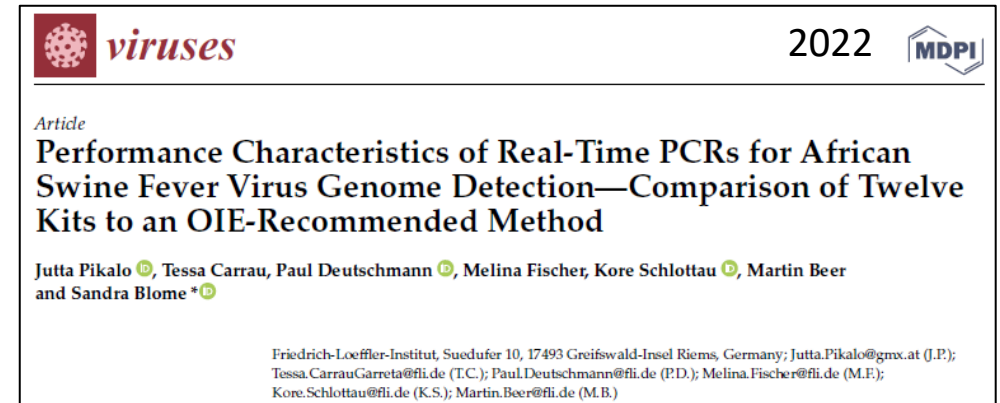
Carlson *et al.* 2019 Trans Anim Dis

- Detection of partial gene fragments of the ASFV genome (eg *B646L* gene encoding p72)
- **Rapid** (~1-2 hrs) and highly sensitive/specific
- **Frontline choice for outbreak investigations and routine diagnostics**
- Can detect virus in absence of infectious virus or when present at low levels
 - Degraded or treated specimens (eg decomposed, pork products)
 - Low/moderate virulence strains



Commercial PCR kits

- Comparisons of performance for different purposes have been reported
 - Range of specimens (field and experimental, wild boar and domestic pigs)
 - Highly virulent or attenuated ASFV, different genotypes
- Generally, comparable levels of sensitivity and specificity, with minor differences between kits and sample types
- For laboratories that do not have capacity to maintain in-house tests, commercial kits are a **viable option**
 - Ensure kit chosen has been validated!





PCR detection of ASFV variants

- **Naturally occurring** lower virulence isolates of **genotype II** ASFV reported in China since 2020
 - Encode different combinations of mutations, insertions, and deletions in various genes (e.g. CD2v, MGF)
- Emergence of **low virulent genotype I** reported in China ('21), associated with chronic ASF
- Emergence of **recombinant genotype I/II** reported in China ('21-'22), Vietnam and Russia ('23), now dominant in Vietnam (~83%)
- Several multiplex qPCR tests reported to detect and differentiate...

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 Zhang¹, Lulu Wang¹, Wenqing Wang¹, Lianyu Huang¹, Fei Xi¹, Haoyue Huangfu¹, Ghebremedhin Tsegay¹, Hong Huo¹, Jianhong Sun¹, Zhijun Tian¹, Wei Xia¹, Xuewu Yu², Fang Li¹, Renqiang Liu¹, Yuntao Guan¹, Dongming Zhao^{1*} & Zhigao Bu^{1*}

¹State 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; ²College of Animal Science and Technology, Inner Mongolia University for Nationalities, Tongliao 028000, China

2021

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



ORIGINAL ARTICLE

OPEN ACCESS Check for updates

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

nature communications

Article

<https://doi.org/10.1038/s41467-023-38868-w>

Highly lethal genotype I and II recombinant African swine fever viruses detected in pigs

Received: 19 October 2022

Accepted: 11 May 2023

Published online: 29 May 2023

Dongming Zhao^{1,3}, Encheng Sun^{1,3}, Lianyu Huang^{1,3}, Leilei Ding^{1,3}, Yuanmao Zhu^{1,3}, Jiwen Zhang¹, Dongdong Shen¹, Xianfeng Zhang¹, Zhenjiang Zhang¹, Tao Ren¹, Wan Wang¹, Fang Li¹, Xijun He¹ & Zhigao Bu^{1,2}

DISPATCHES

Detection of Recombinant African Swine Fever Virus Strains of p72 Genotypes I and II in Domestic Pigs, Vietnam, 2023

Van Phan Le, Van Tam Nguyen, Tran Bac Le, Nguyen Tuan Anh Mai, Viet Dung Nguyen, Thi Tam Than, Thi Ngoc Ha Lai, Ki Hyun Cho, Seong-Keun Hong, Yeon Hee Kim, Tran Anh Dao Bui, Thi Lan Nguyen, Daesub Song, Aruna Ambagala

2024

PCR detection of ASFV variants

BMC Veterinary Research
Article in Press
<https://doi.org/10.1186/s12917-026-05585-7>

Establishment of triplex real-time quantitative PCR assay for African swine fever virus genotype I/II recombinants

Received: 14 February 2026
Accepted: 15 May 2026

Zhuyun Sun, Jiezheng Guo, Yao Li, Tong Sun, Jingyi Liu, Yingnan Liu, Yuchen Nan, Hongjun Chen & Chunyan Wu

A duplex fluorescent quantitative PCR assay to distinguish the genotype I, II and I/II recombinant strains of African swine fever virus in China

2024

Zhiqiang Hu^{1,2†}, Ranran Lai^{1†}, Xiaogang Tian¹, Ran Guan^{1,2} and Xiaowen Li^{1,3,4*}

Research Article
2024

Establishment of a Triplex qPCR Assay for Differentiating Highly Virulent Genotype I Recombinant Virus From Low-Virulence Genotype I and Genotype II African Swine Fever Viruses Circulating in China

Leilei Ding,¹ Tao Ren,^{1,2} Guoxia Bing,³ Zhigang Wang,¹ Baoyue Wang,³ Jianqiang Ni,³ Yuliang Liu,³ Rui Zhao,^{1,2} Yuanmao Zhu,¹ Fang Li,¹ Renqiang Liu,¹ Qiang Fu,^{2,4} Zhijun Tian,¹ Zhigao Bu,¹ Encheng Sun^{1,4} and Dongming Zhao^{1,2}

Establishment of a Dual Real-Time PCR Assay for the Identification of African Swine Fever Virus Genotypes I and II in China

Qi Gao^{1,2,3†}, Yongzhi Feng^{1,2†}, Yunlong Yang^{1,3}, Yizhuo Luo^{1,4}, Ting Gong^{1,4}, Heng Wang^{1,2,3}, Lang Gong^{1,2,3}, Guihong Zhang^{1,2,3*} and Zezhong Zheng^{1,2,3*}

2022

1. 2. PCR Protocol: CAHEC Triplex Assay

Zhiliang Wang et al., CAHEC

The protocol described below was developed by the Chinese Animal Health and Epidemiology Center for the multiplex detection of MGF360/505, EP402R (CD2v) and B646L (p72) genes of ASFV belonging to genotype II.

A triplex real-time PCR method to detect African swine fever virus gene-deleted and wild type strains

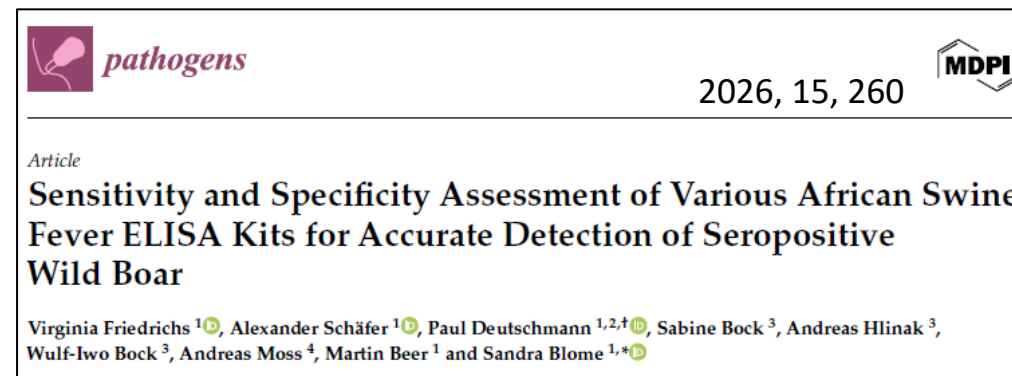
Hao Yang^{1,2}, Zhong Peng^{1,2}, Wenbo Song^{1,2}, Chen Zhang^{1,2}, Jie Fan^{1,2}, Hongjian Chen^{1,2}, Lin Hua^{1,2}, Jie Pei^{1,2,3}, Xibiao Tang², Huanchun Chen^{1,2} and Bin Wu^{1,2*}

2022

- Surveillance for circulating variants and recombinants
- Validation ongoing

Serological testing: pivot

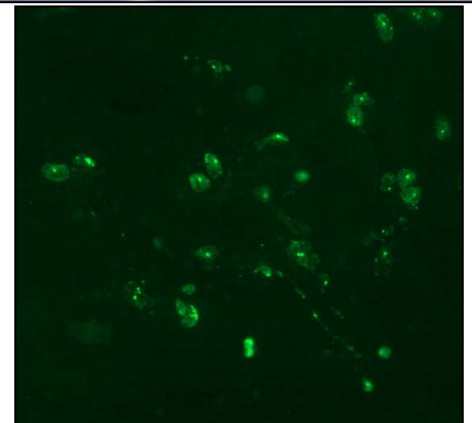
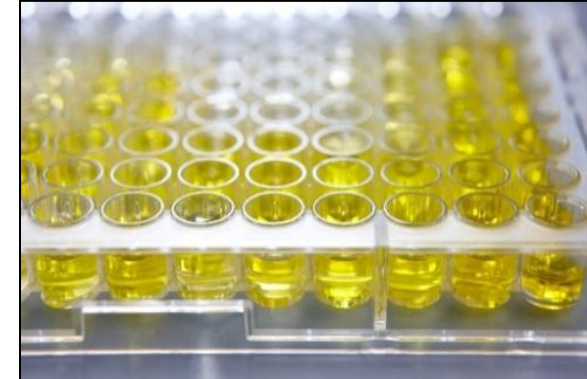
- In areas where **attenuated and lower virulence** viruses circulate, serological testing is recommended for identifying evidence of previous exposure to ASFV in animals experiencing **subacute, chronic or subclinical infections**, or those that have recovered
 - Natural variants, evolved over time
 - Derived from illegal vaccines
- Several commercial and in-house serological test options available for various scenarios





Antibody detection: general considerations

- Current serological tests **cannot differentiate** between natural infected and vaccinated animals
 - No serological DIVA markers in currently licenced vaccines
 - where **vaccines in use**, the *presence of antibodies indicates recent or past infection, or vaccination*
- Recommended serology tests are:
 - **ELISA**, as a screening tool
 - **Indirect fluorescence/peroxidase antibody test (IFA/IPX)** or **immunoblot (IB)** for confirmation





DIVA* PCR

**Differentiating Infected from Vaccinated Animals*

- In the absence of serological DIVA tests, DIVA PCRs are currently the only option
- DIVA assays reported for USDA MLVs (Velazquez-Salinas et al., 2021)
- Multiplex PCRs also reported for differentiating wildtype & vaccine strains
→ Differential detection of p72 and deleted gene(s) or reporter genes (EGFP, mCherry)
- Limited to effective use when MLV is actively replicating in the vaccinated pig

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

2021

Lauro Velazquez-Salinas^{1,2*}, Elizabeth Ramirez-Medina¹, Ayushi Rai^{1,3}, Sarah Pruitt¹, Elizabeth A. Vuono^{1,4}, Nallely Espinoza¹, 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,



Journal of Animals

Article

The Development of a Multiplex Real-Time Quantitative PCR Assay for the Differential Detection of the Wild-Type Strain and the MGF505-2R, EP402R and I177L Gene-Deleted Strain of the African Swine Fever Virus

2022

Kang Zhao^{1,*}, Kaichuang Shi^{1,2,*}, Qingan Zhou^{2,†}, Chenyong Xiong¹, Shenglan Mo², Hongjin Zhou¹, Feng Long², Haina Wei², Liping Hu² and Meilan Mo^{1,*}



Contents lists available at ScienceDirect

Journal of Virological Methods

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



2022

Development of a triplex real-time PCR assay for detection and differentiation of gene-deleted and wild-type African swine fever virus

Yanxing Lin, Chenfu Cao, Weijun Shi, Chaohua Huang, Shaoling Zeng, Jie Sun, Jiang Wu, Qunyi Hua*

A Triplex PCR Method for Distinguishing the Wild-Type African Swine Fever Virus From the Deletion Strains by Detecting the Gene Insertion

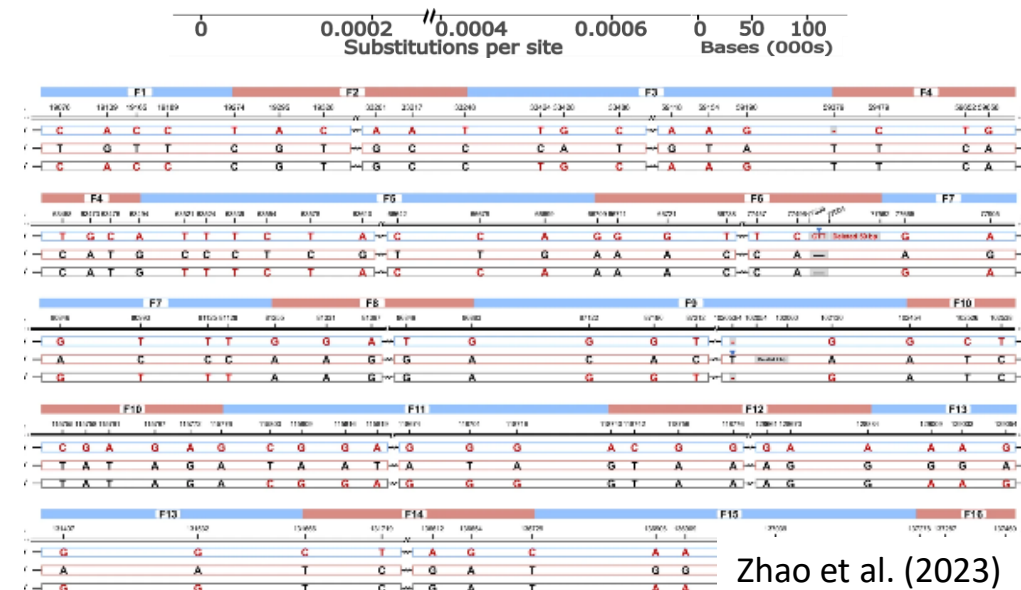
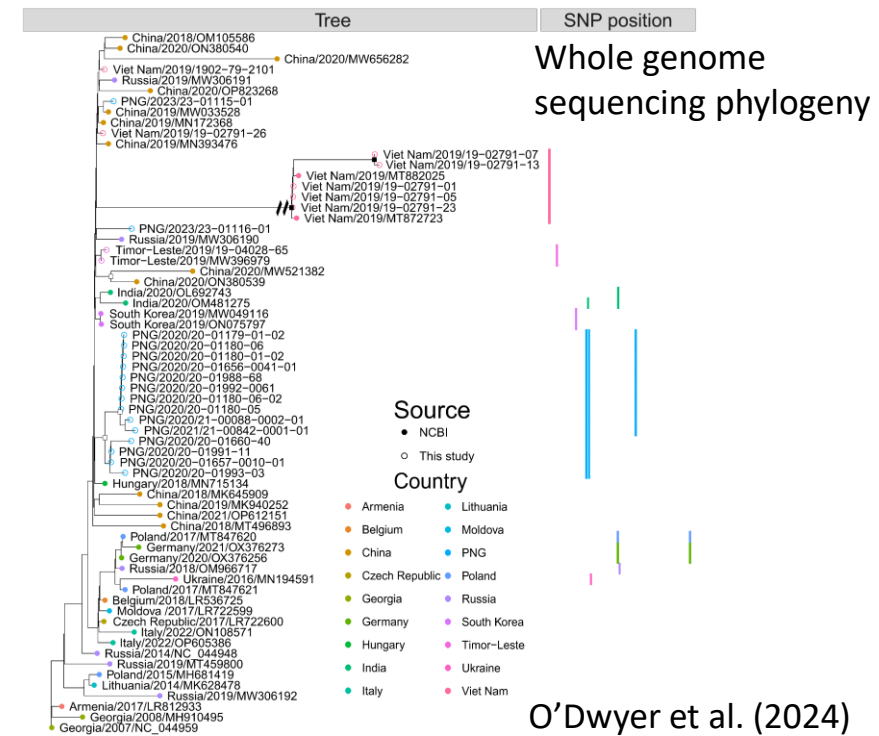
2022

Zhao Huang^{1,2†}, Zhiying Xu^{1,2†}, Haoxuan Cao^{1,2}, Fanliang Zeng^{1,2}, Heng Wang^{1,2,3}, Lang Gong^{1,2}, Shengxun Zhang⁴, Sen Cao⁵, Guihong Zhang^{1,3,6,7*} and Zezhong Zheng^{1,3*}



Genomics and sequencing

- Essential for understanding diversity of genetic variants, their distribution, spread & prevalence
- Informs diagnostics, vaccine development, design of vaccine field evaluations and post vaccination monitoring
- Individual gene targets (e.g. *B602L*) lack sensitivity
- Whole genome sequencing increasingly favoured to provide high resolution analysis for molecular epidemiology and characterization of recombinant genomes





Genomics and sequencing

- Initial whole genome sequencing (WGS) used Illumina sequencing methodologies
- WGS is becoming more accessible through the availability of relatively inexpensive approaches, e.g. nanopore sequencing of tiled amplicons using MinION



RESEARCH ARTICLE

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2023

African swine fever virus – variants on the rise

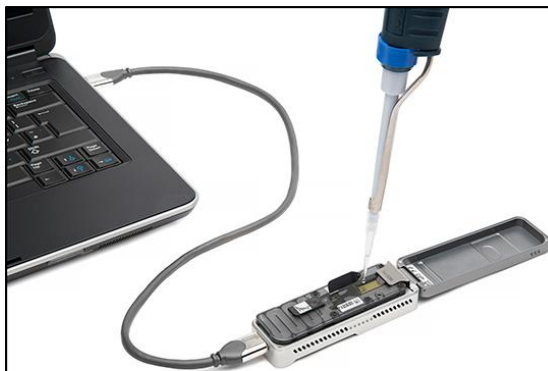
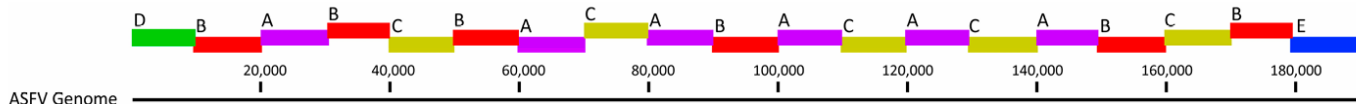
Jan H. Forth^{a*}, Sten Calvelage^{a*}, Melina Fischer^a, Jan Hellert^b, Julia Sehl-Ewert^c, Hanna Roszyk^a, Paul Deutschmann^a, Adam Reichold^d, Martin Lange^d, Hans-Hermann Thulke^d, Carola Sauter-Louis^e, Dirk Höper^a, Svitlana Mandyhra^f, Maryna Sapachova^f, Martin Beer^{g†} and Sandra Blome^{a†}

Research Article

2024

Emergence of Microvariants of African Swine Fever Virus Genotype II in the Asia-Pacific

James O'Dwyer¹, Hung Vo Van², Nguyen Thanh Phuong², Patrick Mileto¹, Orlando Mercado³, Felisiano da Conceição⁴, Joanita Bendita da Costa Jong⁴, Ilagi Puana³, Matthew J. Neave¹ and David T. Williams¹



Oxford Nanopore
Technologies



MINION: PROTOCOL

PCR tiling of African Swine Fever (ASF) virus (SQK-LSK109 with EXP-NBD104 or EXP-NBD114)

V ASFV_9159_v109_revE_18May2022

For Research Use Only

Protocol developed by Amanda Warr *et al.*, 2021



pathogens

2025 MDPI

Article

Targeted Whole Genome Sequencing of African Swine Fever Virus and Classical Swine Fever Virus on the MinION Portable Sequencing Platform

Chester D. McDowell¹, Taeyong Kwon¹, Patricia Assato¹, Emily Mantlo¹, Jessie D. Trujillo¹, Natasha N. Gaudreault¹, Leonardo C. Caserta², Igor Morozov¹, Jayme A. Souza-Neto^{1,3}, Roman M. Pogranichniy^{1,3}, Diego G. Diel² and Juergen A. Richt^{1,4}



Take home messages

- **PCR remains the frontline test** for outbreak investigations and routine diagnostics; however, **antibody tests also now recommended** (e.g. for lower virulence variants, chronic ASF)
- **Range of variants now circulating in the SE Asian region**
 - Led to a proliferation of assay development to keep pace
 - Valuable tools for surveillance
 - Validation of new tests critical (e.g. via WOAHP validation pathway)
- **Continued genomic surveillance is critical** for understanding circulating types and genetic diversity of ASFV in the region
 - Promising new and more affordable approaches



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