African Swine Fever
Reference Laboratory Network

World Organisation
for Animal Health
Founded as OIE

FAO – WOAH Guidelines on ASF diagnostics and Pen-side tests

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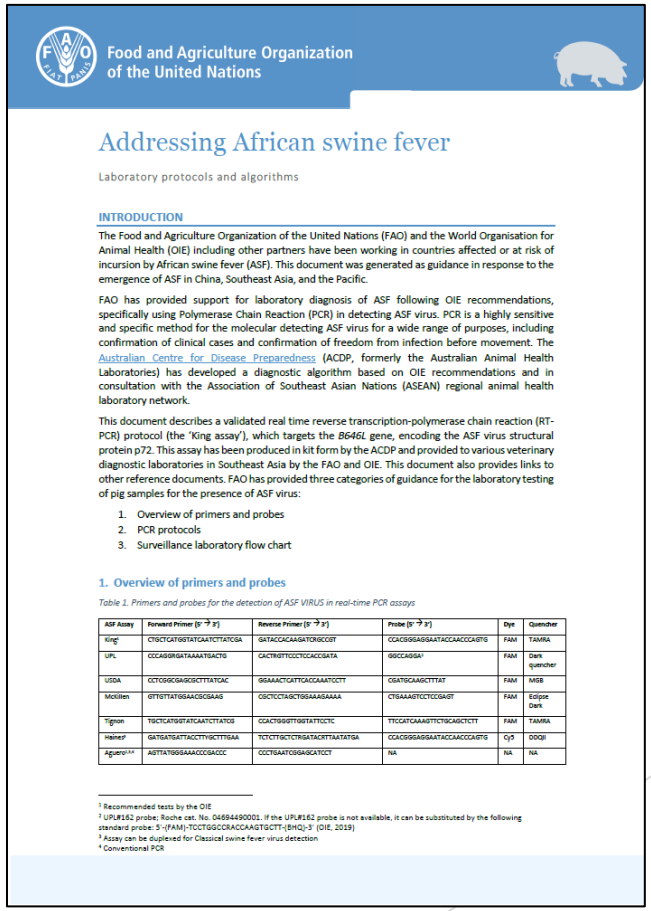
Addressing ASF: Laboratory protocols and algorithms

- Published by the FAO in 2020.
- Basic PCR guideline with laboratory testing algorithm for ASFV
  - PCR protocol for King assay (WOAH-recommended)
  - Test worksheet
- Since then, lower virulence variants and new genotypes have emerged



Food and Agriculture Organization of the United Nations

Addressing African swine fever

Laboratory protocols and algorithms

INTRODUCTION

The Food and Agriculture Organization of the United Nations (FAO) and the World Organisation for Animal Health (OIE) including other partners have been working in countries affected or at risk of incursion by African swine fever (ASF). This document was generated as guidance in response to the emergence of ASF in China, Southeast Asia, and the Pacific.

FAO has provided support for laboratory diagnosis of ASF following OIE recommendations, specifically using Polymerase Chain Reaction (PCR) in detecting ASF virus. PCR is a highly sensitive and specific method for the molecular detecting ASF virus for a wide range of purposes, including confirmation of clinical cases and confirmation of freedom from infection before movement. The Australian Centre for Disease Preparedness (ACDP, formerly the Australian Animal Health Laboratories) has developed a diagnostic algorithm based on OIE recommendations and in consultation with the Association of Southeast Asian Nations (ASEAN) regional animal health laboratory network.

This document describes a validated real time reverse transcription-polymerase chain reaction (RT-PCR) protocol (the 'King assay'), which targets the *B646L* gene, encoding the ASF virus structural protein p72. This assay has been produced in kit form by the ACDP and provided to various veterinary diagnostic laboratories in Southeast Asia by the FAO and OIE. This document also provides links to other reference documents. FAO has provided three categories of guidance for the laboratory testing of pig samples for the presence of ASF virus:

1. Overview of primers and probes
2. PCR protocols
3. Surveillance laboratory flow chart

1. Overview of primers and probes

Table 1. Primers and probes for the detection of ASF VIRUS in real-time PCR assays

ASF Assay	Forward Primer (5'→3')	Reverse Primer (5'→3')	Probe (5'→3')	Dye	Quencher
King[1]	CTGCTCATGGTATCAATCTTATCGA	GATACCACAAGATCRGCCGT	CCACGGGAGGAATACCAACCCAGTG	FAM	TAMRA
UPL	CCCAGGRGATAAAATGACTG	CACTRGTTCCCTCCACCGATA	GGCCAGGA[2]	FAM	Dark quencher
USDA	CCTCGGCGAGCGCTTTATCAC	GGAAACTCATTCACCAAATCCTT	CGATGCAAGCTTTAT	FAM	MGB
McKillen	GTTGTTATGGAACGCGAAG	CGCTCCTASCTGGAAAGAAAA	CTGAAAGTCCTCCGAGT	FAM	Eclipse Dark
Tignon	TGCTCATGGTATCAATCTTATCG	CCACTGGGTTGGTATTCCTC	TTCCATCAAAGTTCTGCAGCTCTT	FAM	TAMRA
Haines[3]	GATGATGATTACCTTYGCTTTGAA	TCTCTTGCTCTRGATACRTTAATATGA	CCACGGGAGGAATACCAACCCAGTG	Cy5	DDQII
Agüero[3,4]	AGTTATGGGAAACCCGACCC	CCCTGAATCGGAGCATCCT	NA	NA	NA

[1] Recommended tests by the OIE
[2] UPL#162 probe; Roche cat. No. 04694490001. If the UPL#162 probe is not available, it can be substituted by the following standard probe: 5'-(FAM)-TCCTGGCCRACCAAGTGCTT-(BHQ)-3' (OIE, 2019)
[3] Assay can be duplexed for Classical swine fever virus detection
[4] Conventional PCR

Food and Agriculture Organization of the United Nations

Emergence of naturally attenuated variants in China

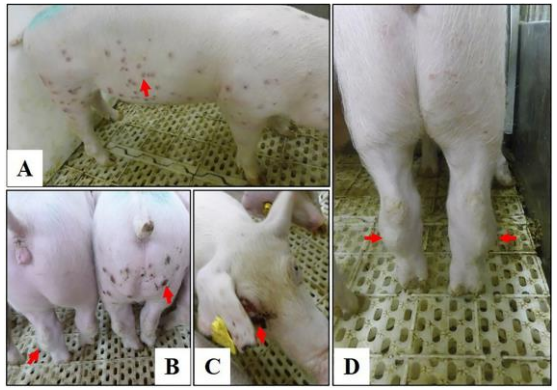
- In 2020, virus isolates characterized as genotype II, with various changes in genomes (mutations, deletions, insertions, or short-fragment replacement)
- 11 isolates had non-haemadsorbing phenotype
  - Deletions or mutations in the *EP402R* gene (CD2v protein)
  - Two isolates tested *in vivo* had lower virulence, but highly transmissible
  - High dose: partially lethal (50-75%), caused **acute or sub-acute** ASF
  - Low dose: non-lethal, **sub-acute or chronic disease**, and persistent infection

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

Sun Encheng[1], Zhang Zhenjiang[1], Wang Zilong[2], He Xijun[2], Zhang Xianfeng[2], Wang Lulu[2], Wang Wenqing[1], Huang Lianyu[1], Xi Fei[1], Huangfu Haoyue[1], Tsegay Ghebremedhin[1], Huo Hong[1], Sun Jianhong[1], Tian Zhijun[1], Xia Wei[1], Yu Xuewu[3], Li Fang[2], Liu Renqiang[2], Guan Yuntao[1], Zhao Dongming[4] and Bu Zhigao[5]

Emergence of Genotype I in China

- In 2021, detection of Genotype 1 viruses for the first time
  - High levels of genomic similarity to historical isolates from Portugal
  - Non-HAD phenotype, with mutations in the EP402R gene (CD2v)
  - Low virulence, efficient transmissibility
- Caused **mild infection and chronic disease**
  - Paralysis, weight loss, intermittent fever, skin ulcers, arthritis and sporadic deaths, swollen joints, papules on the skin and necrotic lesions



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

EMi Taylor & Francis

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

Reports of chronic ASF in China linked to unlicenced vaccines

HEALTHCARE & PHARMA AUGUST 25, 2020 / 7:59 PM / UPDATED 6 MONTHS AGO

China to probe labs, farms in crackdown on illegal African swine fever vaccines

2 MIN READ

By Reuters Staff

(Reuters) - China has begun a strict crackdown
and use of illegal African swine fever vaccines, t
Agriculture and Rural Affairs said on Tuesday,
evidence of the widespread use of such produ

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New China African Swine Fever Strains Point to Unlicensed Vaccines

HEALTH NEWS JANUARY 22, 2021 / 11:15 AM / UPDATED A MONTH AGO

New China swine fever strains point to unlicensed vaccines

By Dominique Patton

7 MIN READ

BEIJING (Reuters) - A new form of African swine fever identified in Chinese pig farms is most likely caused by illicit vaccines, industry insiders say, a fresh blow to the world's largest pork producer, still recovering from a devastating epidemic of the virus.

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China moves to crack down on illegal ASF vaccines

China is moving against the production and use of illegal African swine fever vaccines after anecdotes emerge of their widespread use.

AFRICAN SWINE FEVER NOTIFIABLE DISEASES DISEASE AND WELFARE

by The Pig Site
26 August 2020, at 9:56am

Vaccine progress: USDA/NAVETCO LAV

- Developed by USDA
  - Recombinant Live attenuated vaccine candidate based on Georgia/07 (G2)
  - Deletion of previously uncharacterised gene (I177R)
  - Protects against virulent ASFV challenge in experimental studies

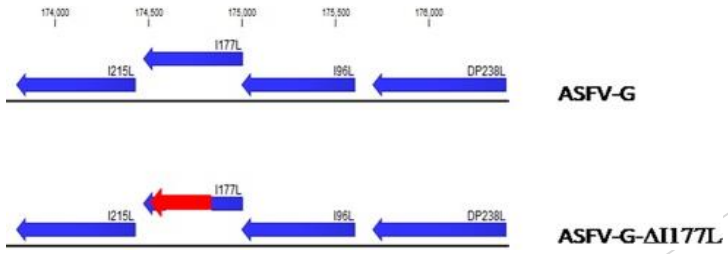
Development of a Highly Effective African Swine Fever Virus Vaccine by Deletion of the I177L Gene Results in Sterile Immunity against the Current Epidemic Eurasia Strain

Manuel V. Borca,[a] Elizabeth Ramirez-Medina,[a,b] Ediane Silva,[a,c] Elizabeth Vuono,[a,d] Ayushi Rai,[a,e] Sarah Pruitt,[a,e] Lauren G. Holinka,[a] Lauro Velazquez-Salinas,[a,c] James Zhu,[a] Douglas P. Gladue[a]

[a]U.S. Department of Agriculture, Agricultural Research Service, Plum Island Animal Disease Center, Greenport, New York, USA

African swine fever virus vaccine candidate ASFV-G-ΔI177L efficiently protects European and native pig breeds against circulating Vietnamese field strain

Xuan Hanh Tran[1] | Thi Thu Phuong Le[1] | Quang Huy Nguyen[1] | Thanh Thuy Do[1] | Van Dung Nguyen[1] | Cyril G. Gay[3] | Manuel V. Borca[2] | Douglas P. Gladue[2]



Updating the ASF Diagnostic Protocols and Algorithm

- With new variants emerging and novel vaccines undergoing trials in Vietnam and China, an updated laboratory guideline is needed
- A new version of the ASF Manual was developed based on WOAH recommendations for laboratory diagnosis of ASF
  - Address **detection of virulent and variant forms** of ASF and the potential future use of authorised LAVs with defined mutations
  - Provide **guidance for early detection** of ASF infection by viruses causing **different clinical forms** of disease (e.g. acute, subacute, chronic and subclinical) → *requires both PCR and serological diagnostic testing for sensitive and accurate lab diagnosis*

Updates to PCR protocols

- Assays selected:
  - Detection of G2 variants with deletions in the MGF360/505 and EP402R genes, associated with sub-clinical and chronic ASF
  - Differential detection of G2 LAV-derived virus containing a deletion in the I177L gene
  - Detection of G1 viruses associated with lower virulence and chronic disease
- All assays have been validated (published or by ASF Reference laboratories)

Triplex PCR assay

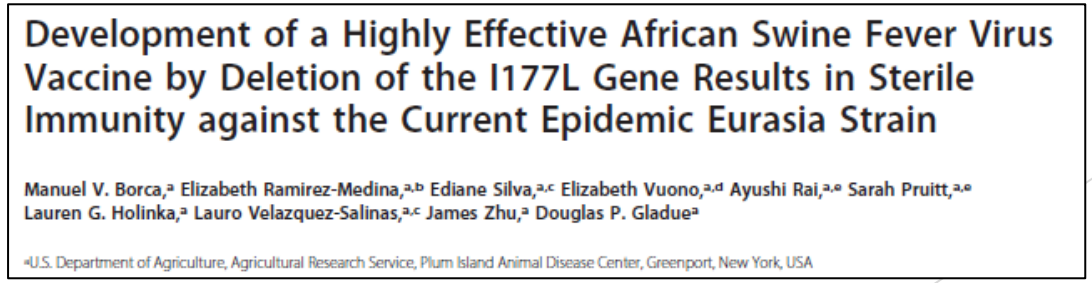
- Developed by the Chinese Animal Health and Epidemiology Center for **multiplex** detection of *MGF360/505*, *EP402R* (CD2v) and *B646L* (p72) genes (Dr Zhiliang Wang)
- DeteValidated using Chinese ASFV isolates belonging to genotype II and *MGF360/505* and *EP402R* (CD2v) genes deletion LAV candidate strains developed by CAHEC
- ct and identify variants containing genome deletions in the *MGF360/505* and *EP402R* (CD2v) genes
- Investigation of subacute and chronic ASF

Interpretation	Test results		
	P72-FAM	CD2v-VIC	MGF-Cy5
ASFV pandemic strain positive	+	+	+
ASFV CD2v gene deletion strain positive	+	-	+
ASFV MGF gene deletion strain positive	+	+	-
ASFV CD2v and MGF gene double deletion strain positive	+	-	-
ASFV negative	-	-	-

Note: "+" means the test is positive;
"-" means the test is negative.

ASFV-G-ΔI177L LAV PCR assay

- Developed and validated by the United States Department of Agriculture (USDA), Agricultural Research Service, at the Plum Island Animal Disease Center (Velazquez-Salinas et al., 2021. Oct 27;8:768869)
- **Genetic DIVA test** for the specific detection of the ASFV-G-ΔI177L LAV candidate
- Use for further molecular characterisation of samples that have tested positive for p72 detection using either the King or the Triplex assay



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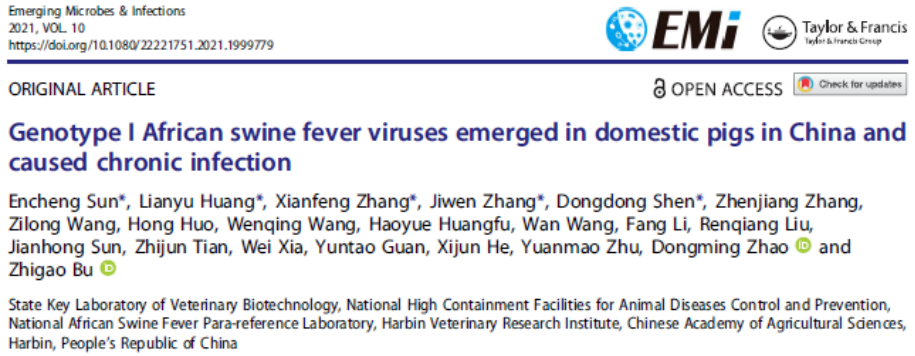
[a]U.S. Department of Agriculture, Agricultural Research Service, Plum Island Animal Disease Center, Greenport, New York, USA

ACDP ASFV Genotype 1 PCR assay

- Targets *505-3R* gene
- Validated using a panel of reference isolates belonging to genotypes 1, 2, 7, 9 and 10, as well as diagnostic specimens
- Should be used for:
  - Investigation of **subacute and chronic ASF**
  - Further molecular characterisation of samples that have tested positive for p72 detection using either the King or the Triplex assay



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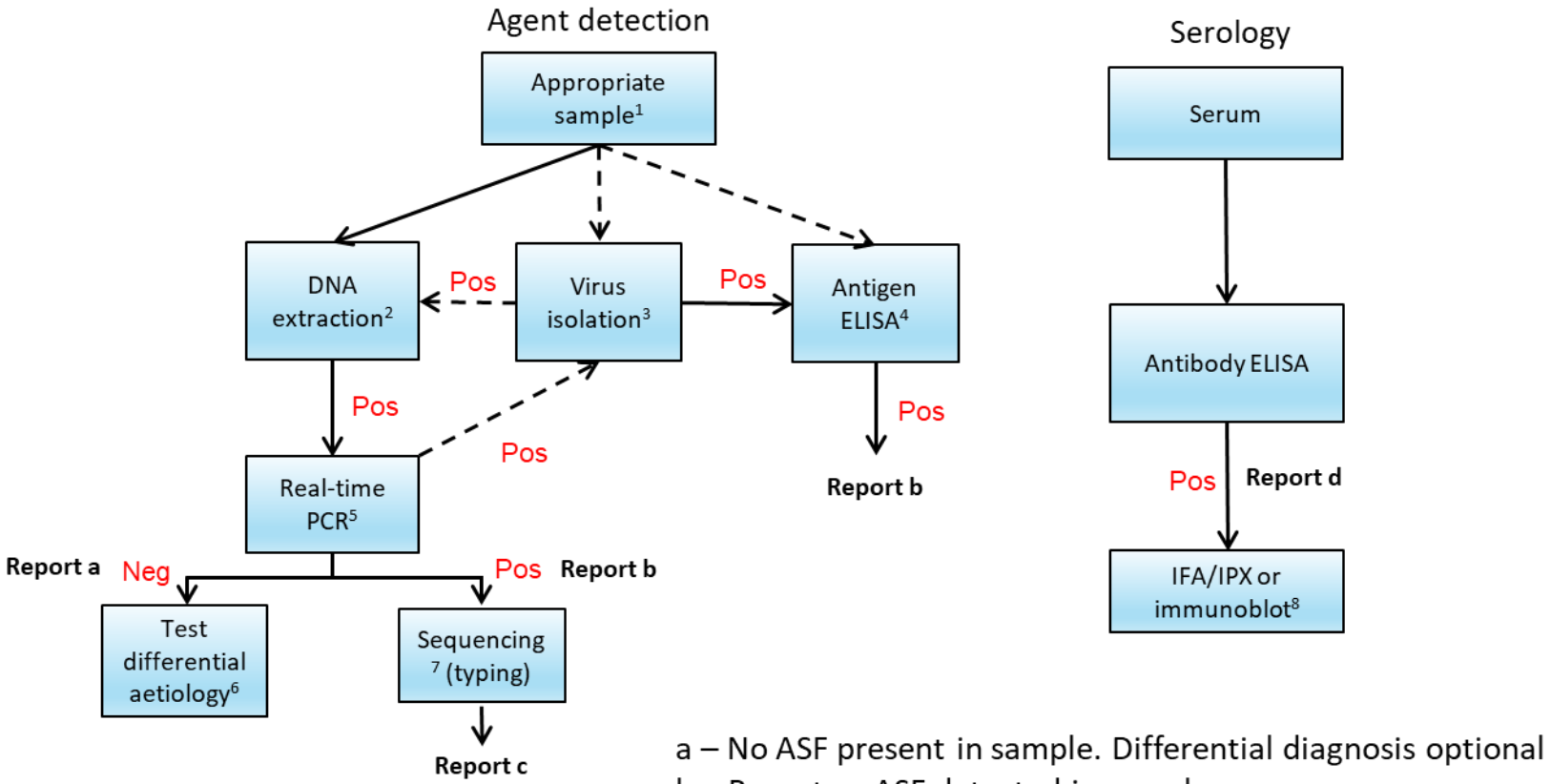
State Key Laboratory of Veterinary Biotechnology, National High Containment Facilities for Animal Diseases Control and Prevention, National African Swine Fever Para-reference Laboratory, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, People's Republic of China

Laboratory algorithm



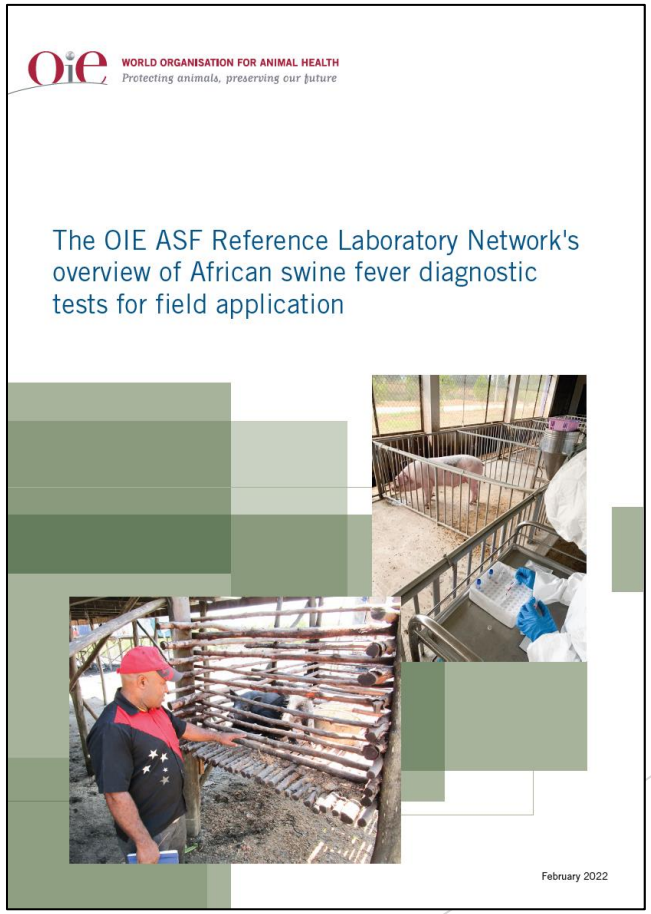
a – No ASF present in sample. Differential diagnosis optional
b – Report as ASF detected in sample
c – Report as ASF detected in sample with genotype/types
d – ASF antibodies detected in sample

ASF Point of Care testing guide

- Several diagnostic platforms available commercially
- The WOAH ASF Reference Laboratory Network has recently drafted an overview of commercially available tests
  - Technical details, costs, advantages vs disadvantages
  - Based on peer-reviewed publications or independent evaluation at Ref lab
  - For ASF diagnosticians, field workers and decision makers

Simple to use
Inexpensive
Low training
Lower Se/Sp
e.g. RATs

More complex to use
Expensive
High training needs
Higher Se/Sp
e.g. PCR/LAMP

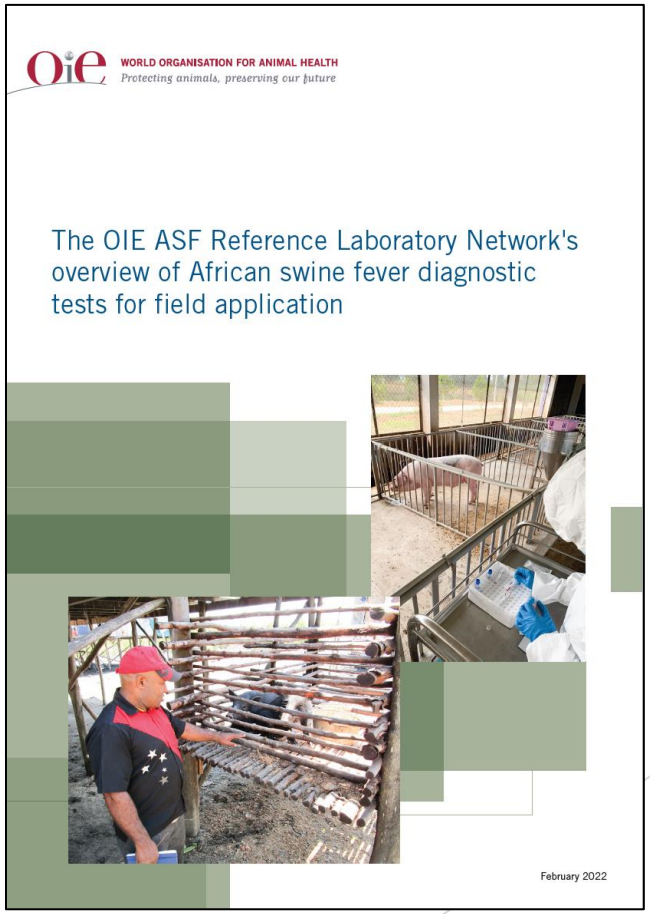


ASF Point of Care testing guide

- Guide is available in English and Spanish, and is being translated into Japanese, Chinese (Mandarin), Bahasa Indonesia, Vietnamese, and Thai

- Free download from:

https://rr-asia.woah.org/en/news/the-oie-asf-reference-laboratory-networks-overview-of-african-swine-fever-diagnostic-tests-for-field-application/



ASF Rapid Tests

Detects virus antigen or antibody in blood samples from infected pigs.
Can be used for disease investigations, as part of ASF surveillance.

Advantages:

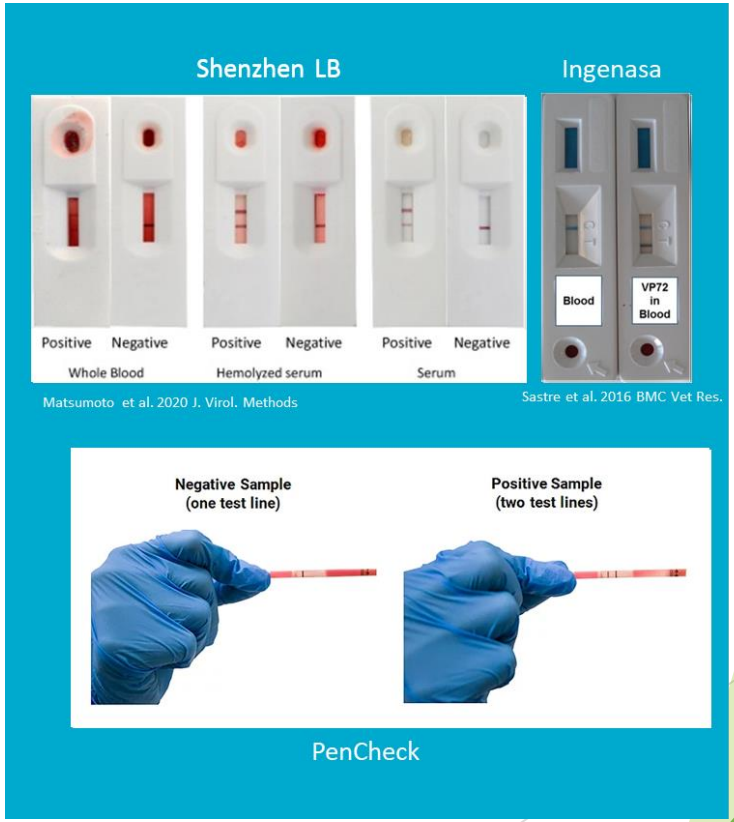
- Use in the field, pen-side
- Simple to use
- Convenient
- Inexpensive
- No instrumentation
- Small volume of sample
- Highly specific

Disadvantages:

- Lower diagnostic sensitivity (Antigen)
  - 60-70%
- Prone to false-negatives
- Can only be used with un-clotted whole blood (Antigen)

Field tests: Antigen detection

- Several commercial options
  - Lateral flow or dip stick
  - Rapid (15-30 mins) and simple to use
  - Inexpensive (~$USD 3-14)
- Typically less sensitive than molecular tests, but can have comparable specificity
- Recommended to:
  - be used on sick or dying pigs (higher levels of viraemia)
  - test samples from more than one sick pig to increase the chances of detecting infection



Field tests: Molecular Tests

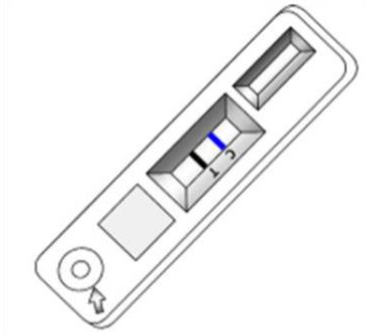
- PCR or isothermal methods
- Sensitivity and specificity comparable to lab-based real-time PCR

  → *detection of ASFV infection (viral DNA) at early stages*
- 0.5-2 hours
- Also used for detection of contaminated carcases, pork and environmental samples at point-of-need (e.g. abattoir, airport, wild boar/feral pig habitats)
- Technically more complex, require much higher level of training and QC/QA, and expensive equipment

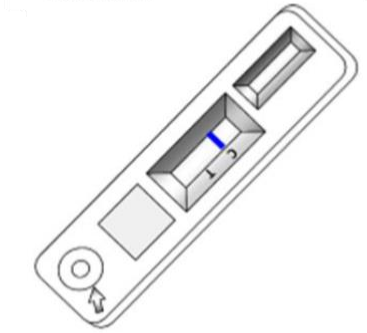
Source: Dr. Ken Inui, FAO

Field tests: Antibody Tests

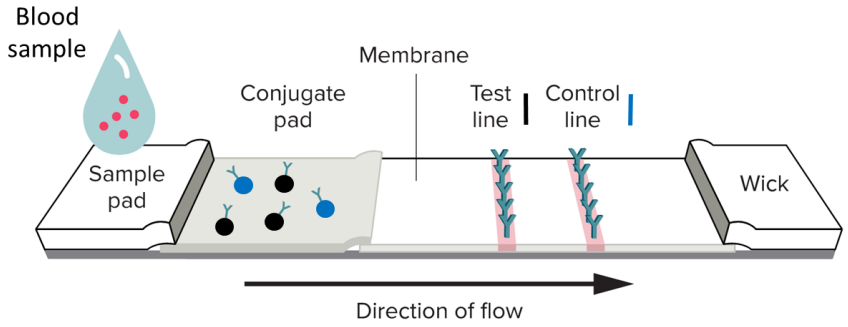
- Lateral flow devices
  - Rapid (15-30 mins) and simple to use
  - Inexpensive (~$USD 5-17)
- Immunochromatographic test
- Comparable diagnostic performance to ELISA
  - Specificity ~100%
  - Sensitivity lower than IPX/IFA



POSITIVE



NEGATIVE



ADAPTED FROM:
SOURCE: D.R. HRISTOV *ET AL* / *SENSORS* 2019

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What to choose?

- Influenced by many factors including costs, ease of use, training requirements, sample type to be tested
- Simple rapid tests may be appropriate for certain situations, such as resource-poor settings
- More advanced molecular platforms may be the test of choice in settings where costs are not a major factor and operators can be confidently trained to a high level of competency
- For some countries, a combination of tests may be employed depending on application and available resources

GF-TADs Africa

GLOBAL FRAMEWORK FOR THE
PROGRESSIVE CONTROL OF
TRANSBOUNDARY ANIMAL DISEASES

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World Organisation
for Animal Health
Founded as OIE

African
Union