



National Updates and Opportunities to strengthen capacities for ASF outbreak management

*Promoting effective surveillance and diagnostics:
Point of care, rapid testing in field practice,
lessons learnt and improving access to diagnostics*

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**African Swine Fever
Reference Laboratory Network**



World Organisation
for Animal Health
Founded as OIE

ASF SGE meeting no. 4

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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
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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 [3' → 5']	Probe [5' → 3']	Dye	Quencher
King ¹	CTCTCATGATGATCAATCTTATCGA	GATACACAGATGTCGGCT	CGCAGGAGGATACCAACCGATG	FAM	TAMRA
UPL	CCGAGGATGATAAATGACTG	CACGTTCTCCACCGATG	GGCCAGAA ²	FAM	Quencher ³
USDA	CTCTGGAGGCGCTTATCAC	GGAAACTGATGACCAATCTCT	CGATGCAAGCTTAT	FAM	NHEX
McIsben	GTFTTATGGAGCGGAG	CGCTCTGACTGGAAGAGAAA	CTGAAGTCTCCGAT ⁴	FAM	Eclipse Dark
Signon	TGCTCATGATGATCAATCTTATCG	CGACTGGTGTGATATTCG	TTCATCAAAAGTCTGAGCTCT	FAM	TAMRA
mainP	GATGATGATCTTCTTCTTGA	TCTTCTCTGATGATGATGATG	CGCAGGAGGATACCAACCGATG	Cy5	DOQB
Agvpr ^{5,6}	AATTATGGAAACCGACCC	CCCTAATGGAGCATCT	NA	NA	NA

¹ Recommended tests by the OIE
² UPL#162 probe: Roche cat. No. 04894490001. If the UPL#162 probe is not available, it can be substituted by the following standard probe: 5'-FAM-TCTCTGCGACCAAGTCTCT-(BHQ)-3' (OIE, 2019)
³ Assay can be duplicated for Classical swine fever virus detection
⁴ Conventional PCR



ASF Point of Care testing guide

- ▶ Several diagnostic platforms available commercially
- ▶ The WOAHS 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/>

Oie WORLD ORGANISATION FOR ANIMAL HEALTH
Protecting animals, preserving our future

The OIE ASF Reference Laboratory Network's
overview of African swine fever diagnostic
tests for field application

February 2022



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

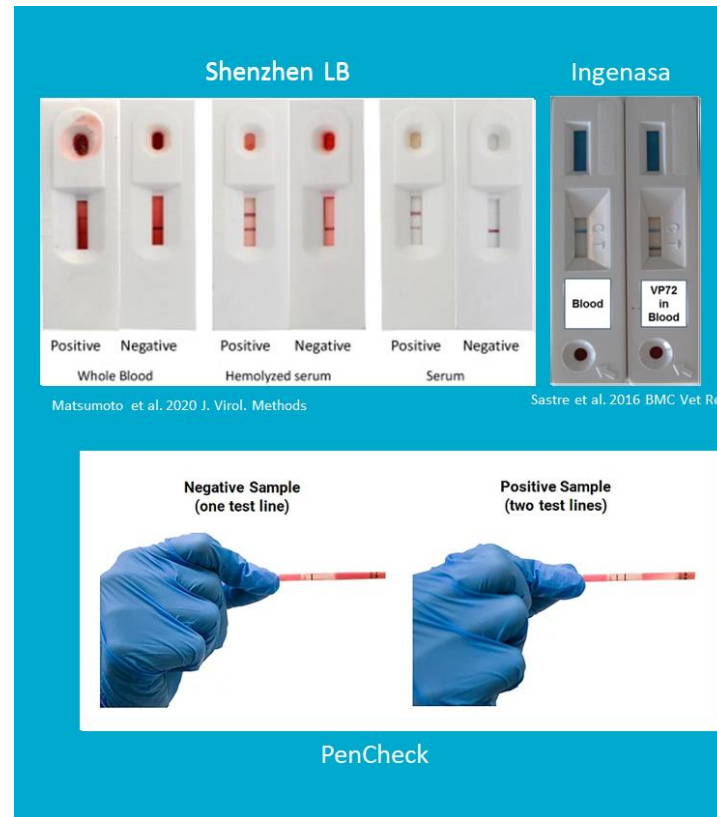
Disadvantages:

- 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 carcasses, 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

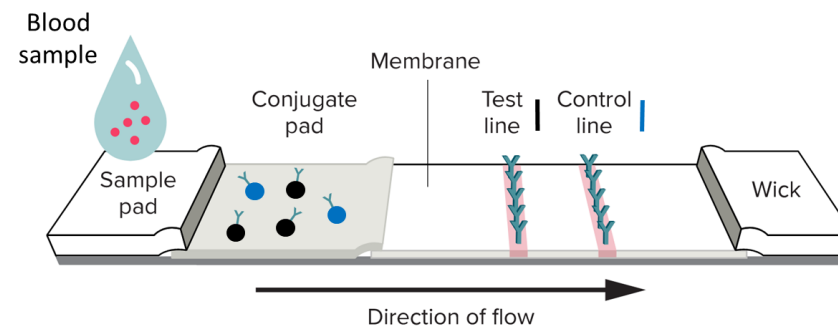
▶ Lateral flow devices

- ▶ Rapid (15-30 mins) and simple to use
- ▶ Inexpensive (~\$USD 5-17)

▶ Immunochromatographic test

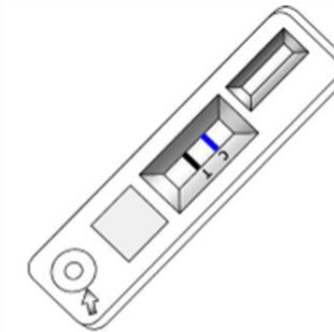
▶ Comparable diagnostic performance to ELISA

- ▶ Specificity ~100%
- ▶ Sensitivity lower than IPX/IFA

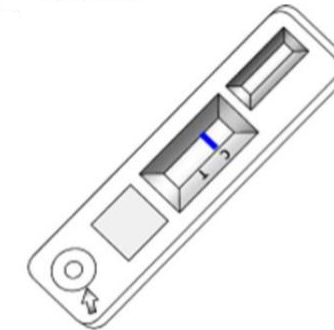


ADAPTED FROM:
SOURCE: D.R. HRISTOV ET AL / SENSORS 2019

KNOWABLE MAGAZINE



POSITIVE



NEGATIVE



Examples of Application

▶ Rapid antigen tests:

- ▶ Outbreak response in Papua New Guinea
- ▶ ASF preparedness for Pacific Island countries (PHAMA Plus)

▶ Rapid antibody tests:

- ▶ Outbreak response and surveillance in Sardinia (moderately virulent virus); *Cappai et al. J Wildlife Dis 53(3):602*

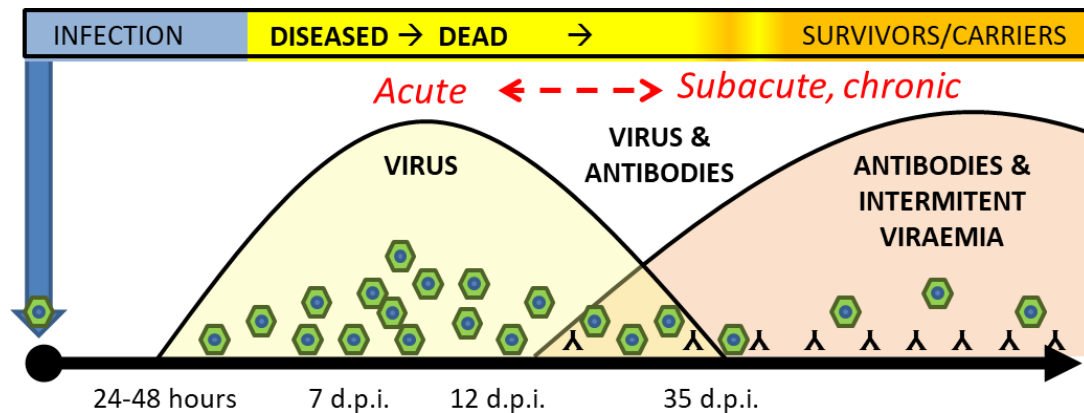
▶ Molecular platforms:

- ▶ POKKIT iiPCR: Used for detection of ASFV in various tissue types by FAO in Southeast Asia, recently evaluated (Tran et al. JAVMA 259:6)
- ▶ Portable real-time PCR: evaluated in Uganda for outbreak testing (*Liu et al. TAD 66(2):908*)



Application in partial culling?

- ▶ Point-of-care tests enable rapid response to outbreaks and control spread of disease in endemic situations.



- ▶ PoC should be applied after laboratory confirmation.
- ▶ PoC should be used to define the status of a herd/group of animals.



Support from the WOAAH/FAO Reference Laboratory

- ▶ The WOAAH/FAO Reference (ARC-OVI) is establishing a stockpile of diagnostic reagents to support the rapid response to ASF outbreaks.
- ▶ Member state will have access to sufficient reagents to perform 500 diagnostic assays. (Real-time PCR, Lateral flow devices, ELISA kits)
- ▶ The type of reagents will depend on the laboratory capacity of the member state.

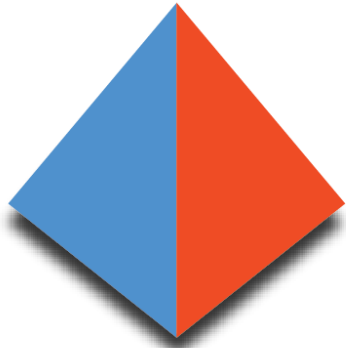
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GF-TADs

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PROGRESSIVE CONTROL OF
TRANSBOUNDARY ANIMAL DISEASES

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