



ASF Diagnostic Tools

ASEAN AFRICAN SWINE FEVER
(ASF) WORKSHOP

David Williams | 2nd-4th May 2023

Australia's National Science Agency





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
 - Sample integrity
 - Prompt submission
 - Documentation and labelling
- Laboratory techniques currently used enable confident diagnosis of ASF across a range of epidemiological situations



Philippines, 2019 (Myra Hosmillo, University of Cambridge)





Field Diagnostics for ASF

- In some settings, timely submission, processing and lab testing of samples is not feasible
- Field diagnostic tests (pen-side, point-of-care/need) are available that **enable rapid response** to outbreaks and control the spread of disease in these situations, e.g.:
 - Outbreak investigation
 - Surveillance (farms, backyard, wildlife)
 - Quarantine stations
 - Live animal markets, abattoir
 - Commercial farm biosecurity

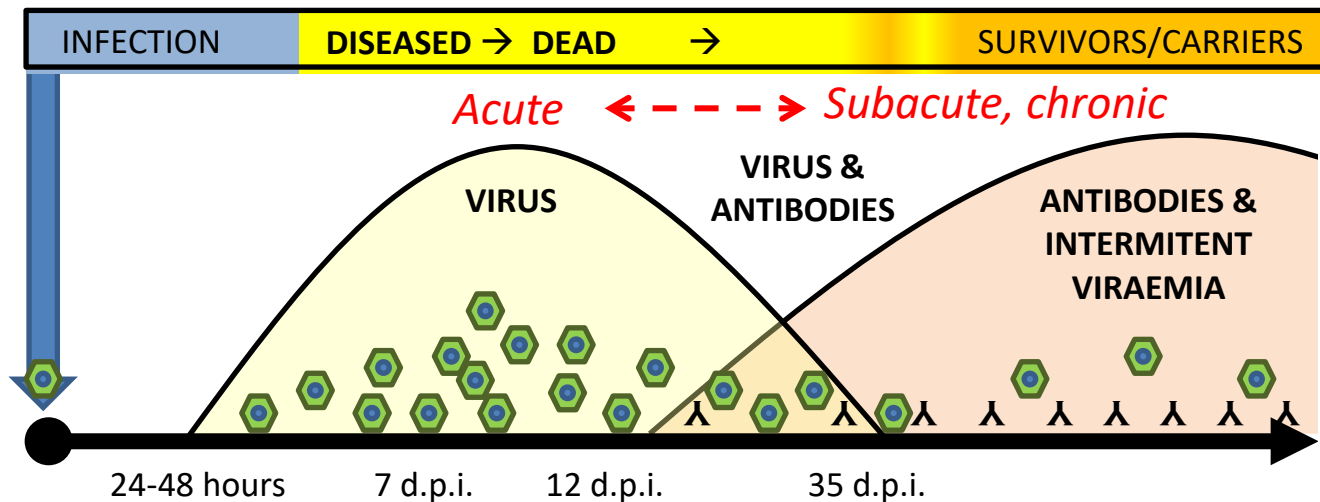


Source: Dr. Ken Inui, FAO



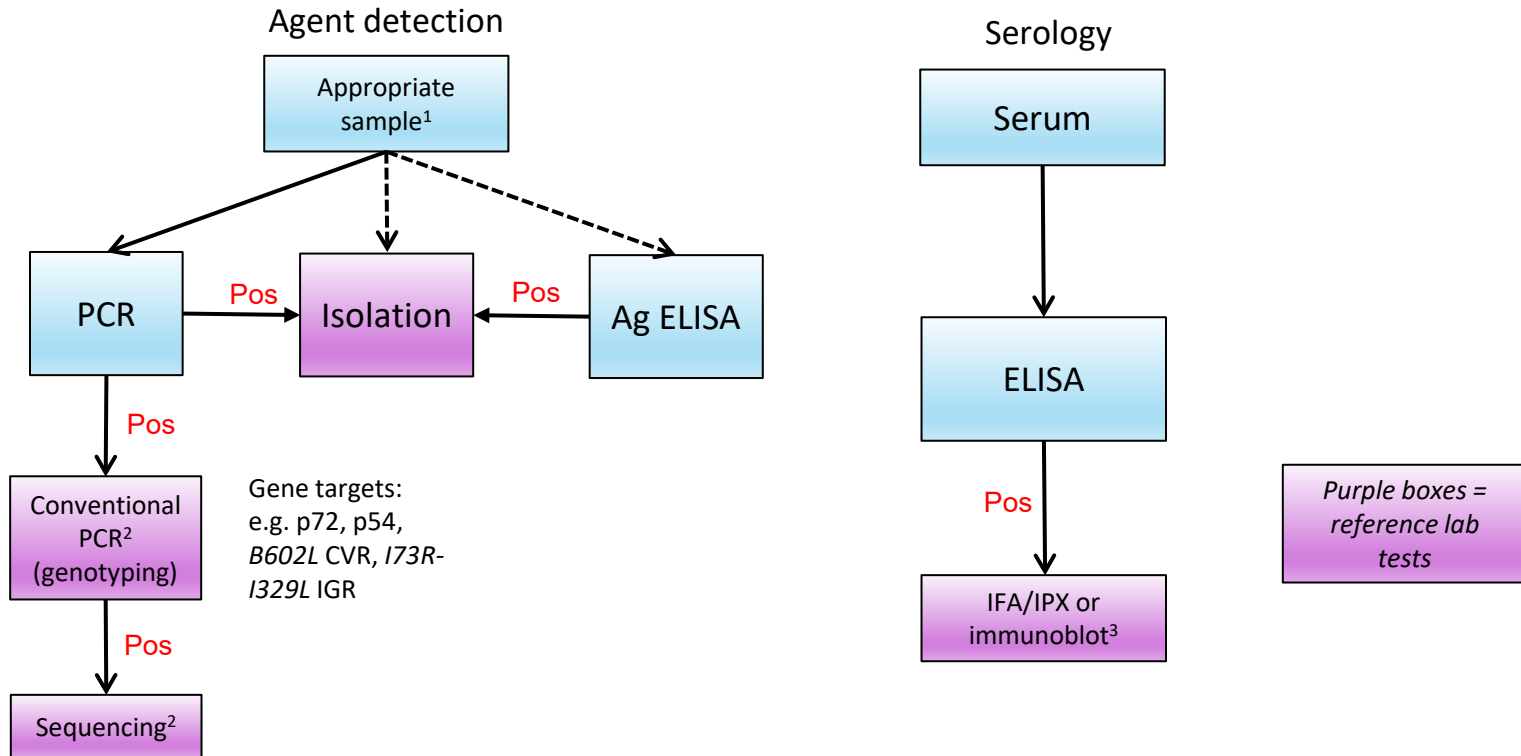
Diagnostics for ASF

- The same principles of lab testing apply to the field
- 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





Laboratory diagnostic algorithm for ASF



1. EDTA blood, spleen, lymph nodes, tonsils, kidneys
2. At start of outbreak/on selected isolates
3. For confirmation or clarification



Updating the Laboratory Algorithm for Asia-Pacific

- New version developed based on WOAH recommendations for laboratory diagnosis, in consultation with WOAH ASF Reference Laboratory Network:
 - Addresses **detection of variant forms of ASF** and potential future use of **authorised LAV vaccines**
 - Provides **guidance for early detection of infection by viruses causing different clinical forms of ASF** (e.g. acute, subacute, chronic and subclinical)
 - **both PCR and serological testing**

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	CTCTCATGSETATCAATCTTATCGA	GATACCAAGATGCRGCGT	CCACGGGAGGAAATACCAACCCAGTGG	FAM	TAMRA
UPL	CCCAGSRGATAAAATGACTG	CACRTTTCCTCCACCAGTA	GGCCAGGA ²	FAM	Dapi quencher
USDA	CTCGGCGAGGCGCTTATCAC	GGAAACTCATTACCAAAATCCCTT	CGATSCAAGCTTAT	FAM	MSB
Mokilen	GTTCCTATGGAACCGGGAAG	CGCTCTACTGCGAAGAAAA	CTGAAAGTCTCCGAGT	FAM	Edipye Quen
Tignon	TTCCTATG@ATCAATCTTATG@	CGACTGG@TTGGTATCTCTC	TTCCATCAAG@TTCGCACTCTT	FAM	TAMRA
Haines ³	GATGATGATTACCTTCTCTTGA	TCTCTGCTCTR@ATACRTTAATATGA	CCACGGGAGGAAATACCAACCCAGTGG	Cy5	DOHQ
Aguero ^{4,4}	AGTTATGGGAAACCCGACCC	CCCTGAATCGAGCATCT	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)-TCTCTGGCCGACCAAGTGGCTT-(BHQ)-3' (OIE, 2019)

³ Assay can be duplicated for Classical swine fever virus detection.

⁴ Conventional PCR



Updated Laboratory Algorithm: PCR

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



Serological testing: pivot

- In ASF endemic areas, where attenuated and lower virulence viruses circulate, serological testing is valuable 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



Field Testing

- The WOAHA 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



- 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/>

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





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
- Ensure test of choice has been validated!

WOAH Reference Laboratory Network

- Members of the core WOAH Reference Laboratory Network:
 - *Onderstepoort Veterinary Institute, South Africa*
 - *Pirbright Institute, United Kingdom*
 - *Centro de Vigilancia Sanitaria Veterinaria (VISAVET), Spain*
 - *CSIRO Australian Centre for Disease Preparedness*
 - *China Animal Health and Epidemiology Center, China*
 - *National Centre for Foreign Animal Disease, Canada*
 - *Foreign Animal Disease Diagnostic Laboratory, USA*



WOAH Reference Laboratories Network

- WOAHA Reference Laboratory Network → Expanded Laboratories Network to include selected national reference or ASF laboratories in:
 - *North and Central America, South America, Africa, Europe, Asia and Oceania*
- Aim of network is to facilitate collaboration between the WOAHA Reference Laboratories and national reference laboratories
- Selection criteria:
 - Recognition as a Regional Reference Laboratory or National Reference Laboratory
 - Actively conducting ASF diagnostics (for disease investigation, surveillance and control, and export certification)
 - Complying with WOAHA Standards for Management and Technical Requirements for Laboratories Conducting Tests for Infectious Disease

Key objectives of the Network



1. To harmonize, standardize and validate ASF diagnostic assays, including assays not currently described in the Terrestrial Manual
2. To facilitate exchange of reference material to be used for internal verification of ASF diagnostic assays
3. To contribute to a worldwide data bank on ASFV genomic data, including the curation of complete genome sequences
4. To provide expertise and training to WOAHA and Member Countries in ASF diagnosis, surveillance and control
5. To support national reference laboratories in ASFV diagnostics by providing scientific and technical expertise
6. To collect, analyse and disseminate epidemiological information on ASF global occurrence and spread, ASF genetic characterization

WOAH Laboratories Network and ASEAN



- As part of the ASEAN ASF Prevention and Control Strategy, the appointment of a ASEAN Reference Laboratory for ASF has been proposed
- We further propose that the ASEAN Reference Laboratory joins the WOAH ASF Reference Laboratories Network
 - To play an active role in delivering network objectives to AMS
 - Aligned and harmonised with WOHAF network objectives and activities for ASEAN ASF Prevention and Control Strategy



Australian Centre for Disease Preparedness

Dr. David Williams

Leader, Diagnosis and Mammalian Infectious Disease Research Group

WOAH Expert at the WOAH Reference Laboratory for African swine fever

t +61 3 5227 5364

e d.williams@csiro.au

