Dr Livio Heath

OIE Expert for African swine fever (Africa)

ASF Diagnostics and Research

Regional training course (Africa)
Import risk analysis for African swine fever
9 November – 14 December 2021

Organisation Mondiale de la Santé Animale
World Organisation for Animal Health
Organización Mundial de Sanidad Animal

The Virus

- ASFV virions have a complex multilayer structure
- More than 50 proteins are included in the virion
- Proteins on surface of virus particle are targets for antibody mediated protection
- The major capsid protein are used in various diagnostic assays

Genome

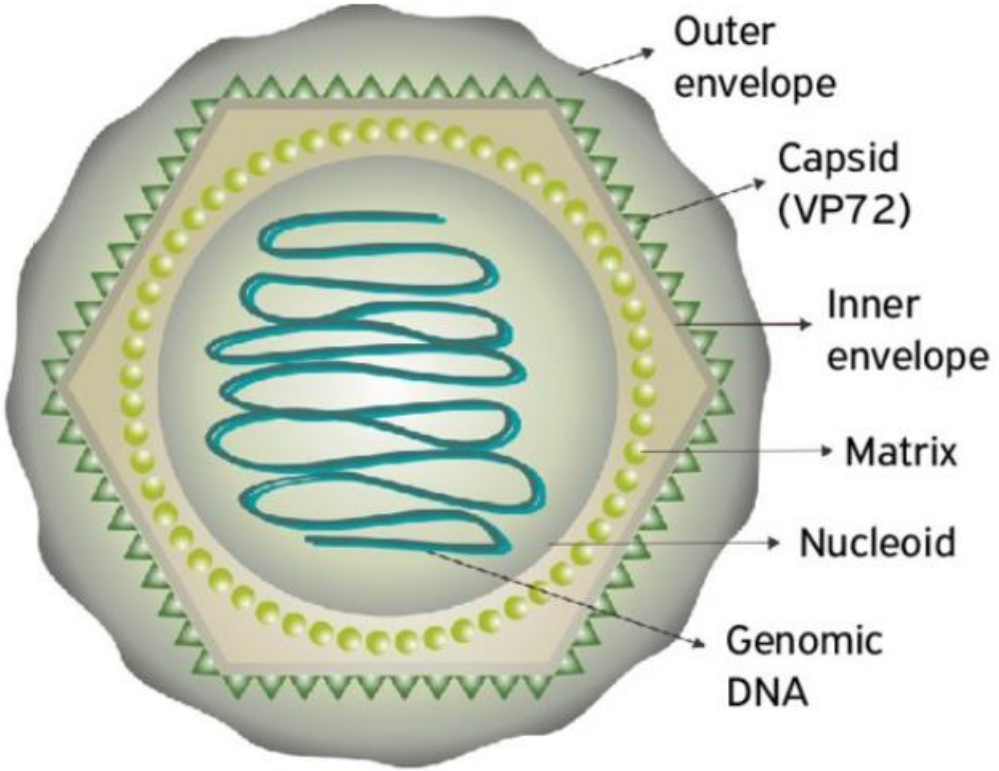
Large double-stranded DNA virus

Genes

Encodes about 151-167 genes

Control

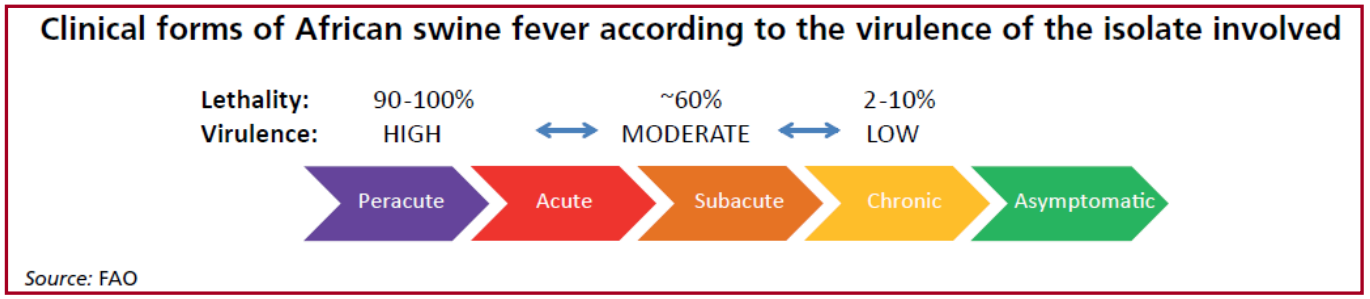
No registered vaccine available.

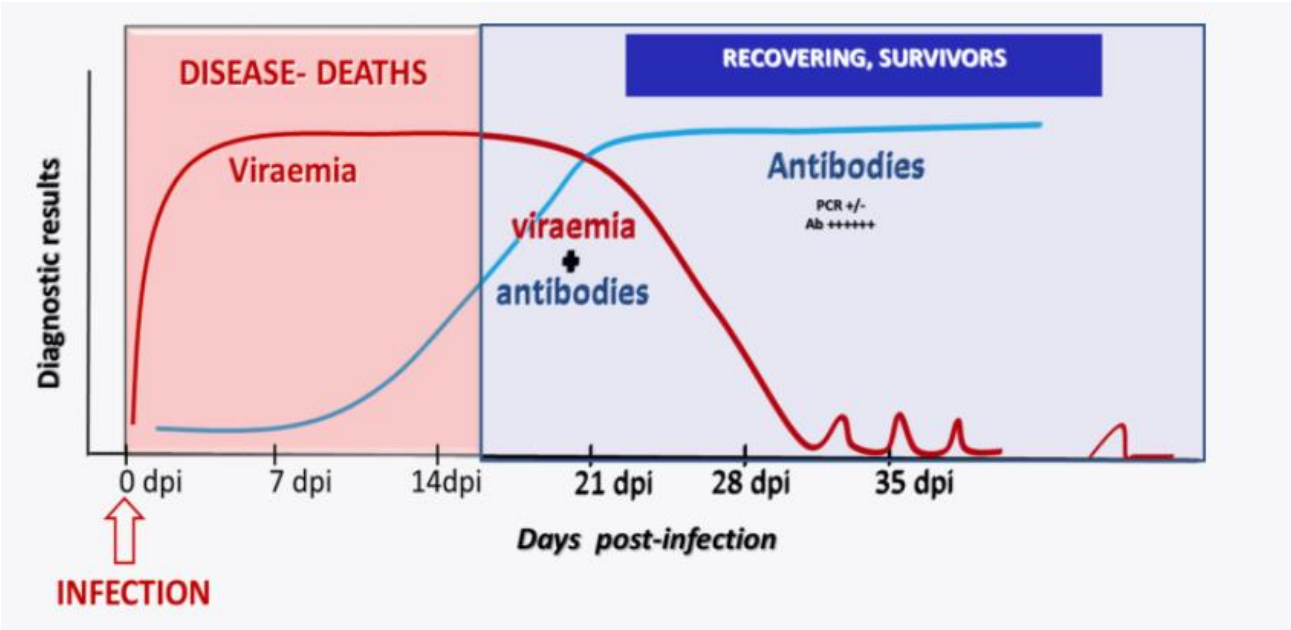


Source: Freitas; Tavares 2016

The Disease

- Acute cases of ASF can result in 100% Mortality
- Some pigs develop subacute or chronic forms of the disease
- Viraemia develops within 2-4 days of infection
- Acutely infected animals often die before developing antibodies





Source: https://asf-referencelab.info/

Diagnosis

Sample Matrixes

- Spleen, lymph nodes, kidneys
- Serum
- Plasma
- EDTA blood
- Meat juice
- Whole blood

Contact the OIE Reference laboratory for assistance and confirmation of test results

Clinical Diagnosis

Clinical signs
Lesions
Sampling and shipment

Molecular Testing

Polymerase Chain Reaction (PCR)
Sequencing
Phylogenetic analysis

Antibody Detection

Competitive ELISA
Immunoblot Assay
Pen-side Antibody test

Clinical Diagnosis

- Pigs are visibly weak with fever and huddle to stay warm
- Bloody diarrhea and disc tint hyperaemic areas on the skin of the neck, chest and extremities.
- Cyanosis at the tips of the ears
- Necrotic lesions on skin of the abdomen, neck and ears

Source: FAO

Clinical Diagnosis

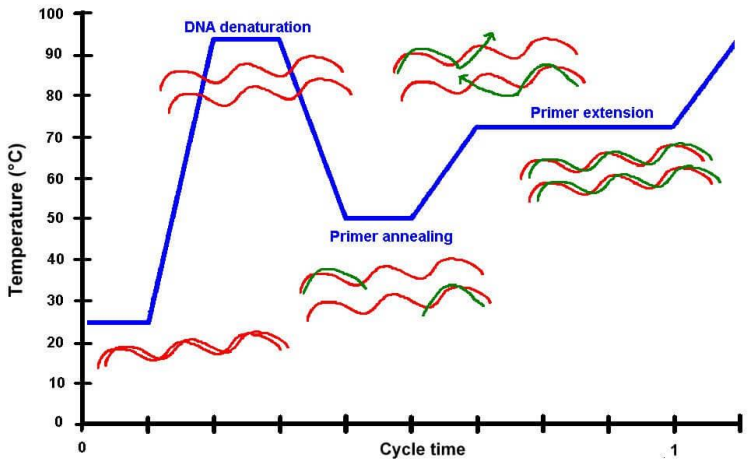
- Froth in the trachea from severe lung oedema
- Haemorrhagic gastohepatic limph nodes
- Haemorrhagic kidney
- Petechiation on the kidney's cortex
- Spleen enlargement

Source: FAO

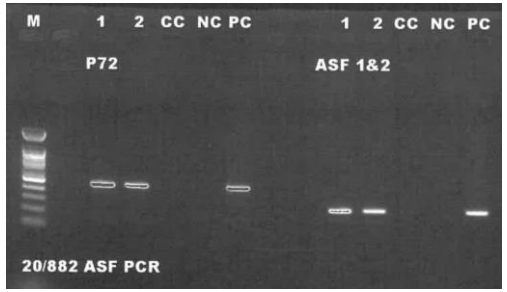
Molecular Testing

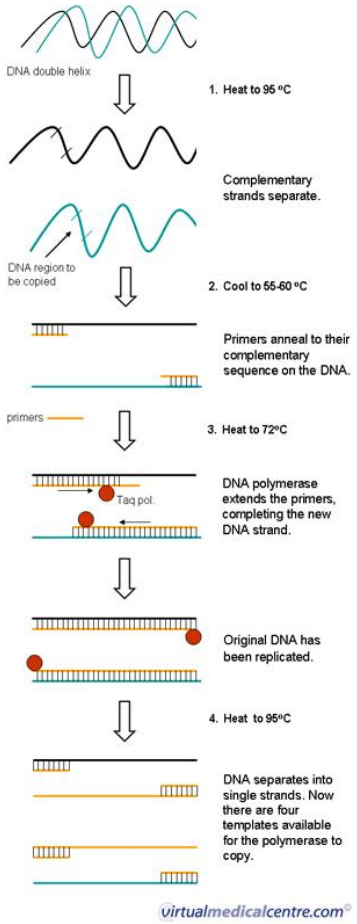
- PCR is used to confirm the suspicion of ASF based clinical signs
- Tissue, whole blood and serum samples can be tested by PCR.
- Convectional PCRs for ASF target a conserved region of the P72 gene
- Sequencing is often used to confirm ambiguous results obtained by conventional PCR.

Conventional PCR



Phylogenetic PCR **Diagnostic PCR**





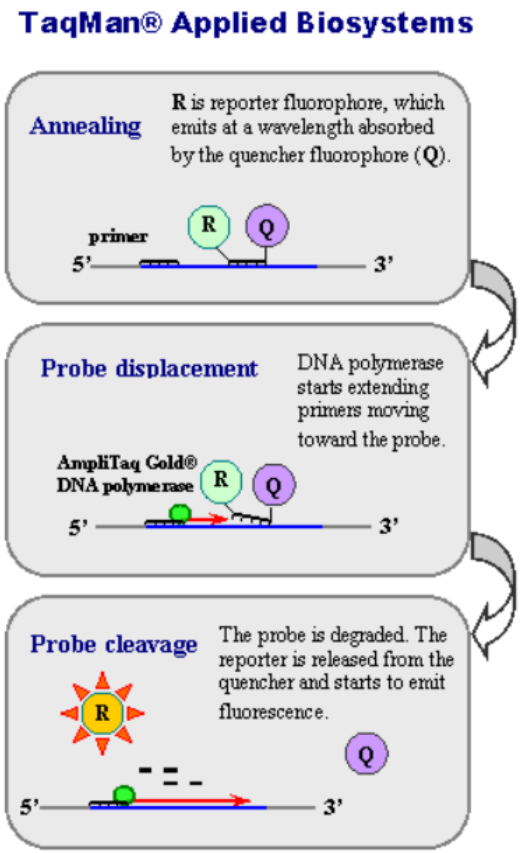
Molecular Testing

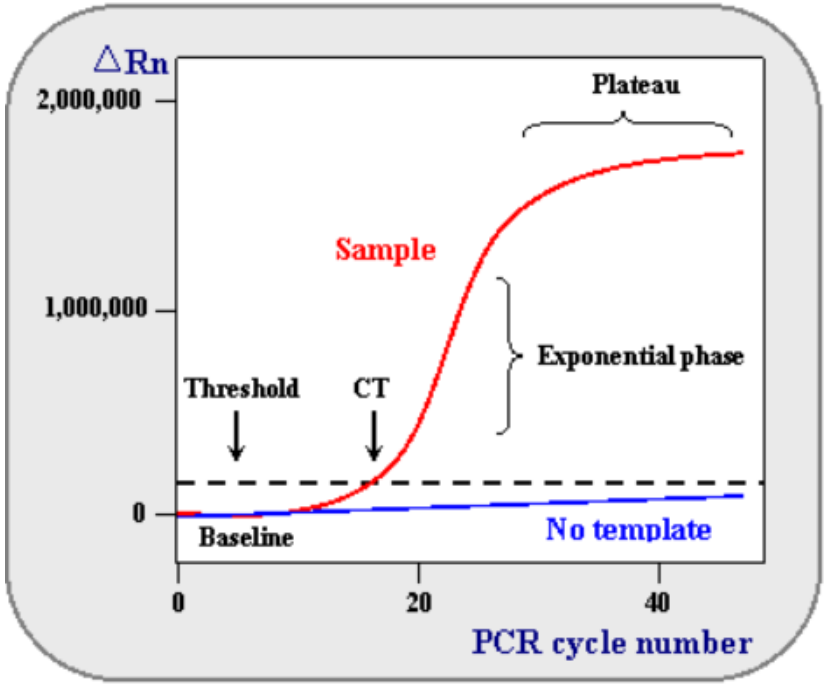
- Real-time PCR has largely replaced conventional PCR as the preferred method for ASF Diagnosis
- Several commercial test kits, based on TaqMan® technology, are available
- Real-time PCR is more sensitive and specific than conventional PCR

Average Specificity - 99,14%

Average Sensitivity - 98,67%

Real-Time PCR





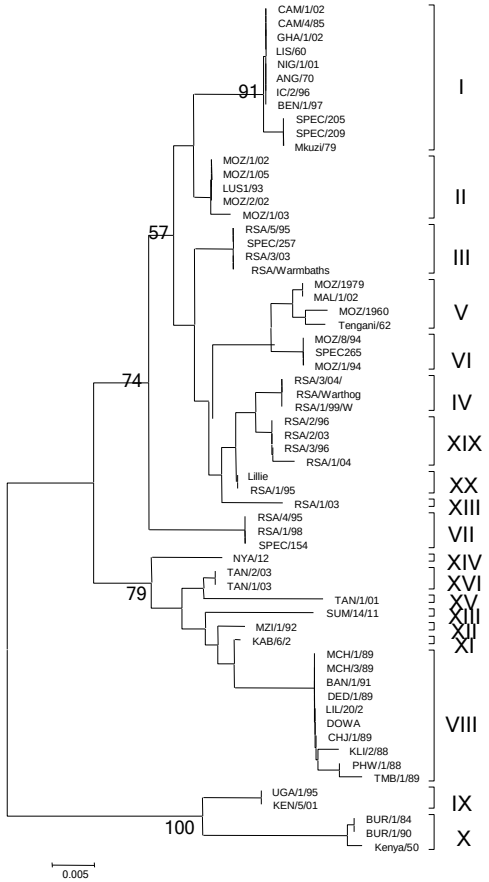
Real Time PCR - Basic simple animation

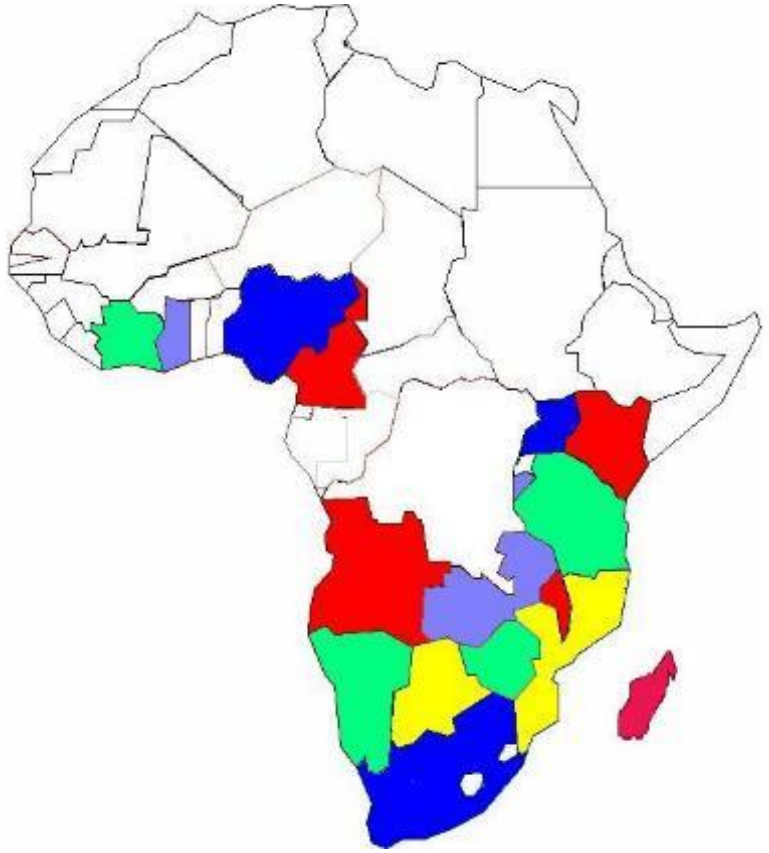
https://www.youtube.com/watch?v=EaGH1eKfvC0

Molecular Testing

- Classifies viruses into genotypes based on the P72 gene sequence
- 24 Genotypes have been described
- Phylogenetic analysis can be used to trace the possible origin of an outbreak and to track the spread
- Phylogenetic analysis should be interpreted together with epidemiological information

Phylogenetic Analysis



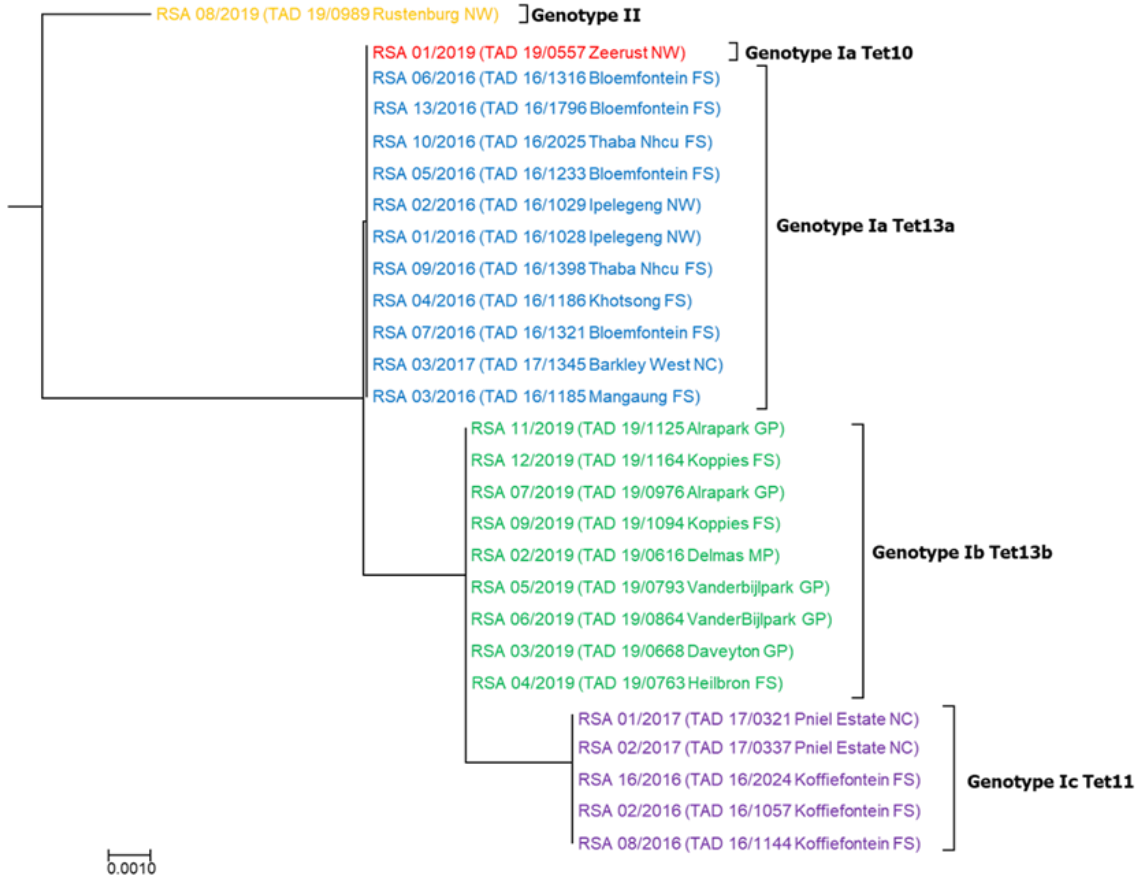


Molecular Testing

ASF in South Africa

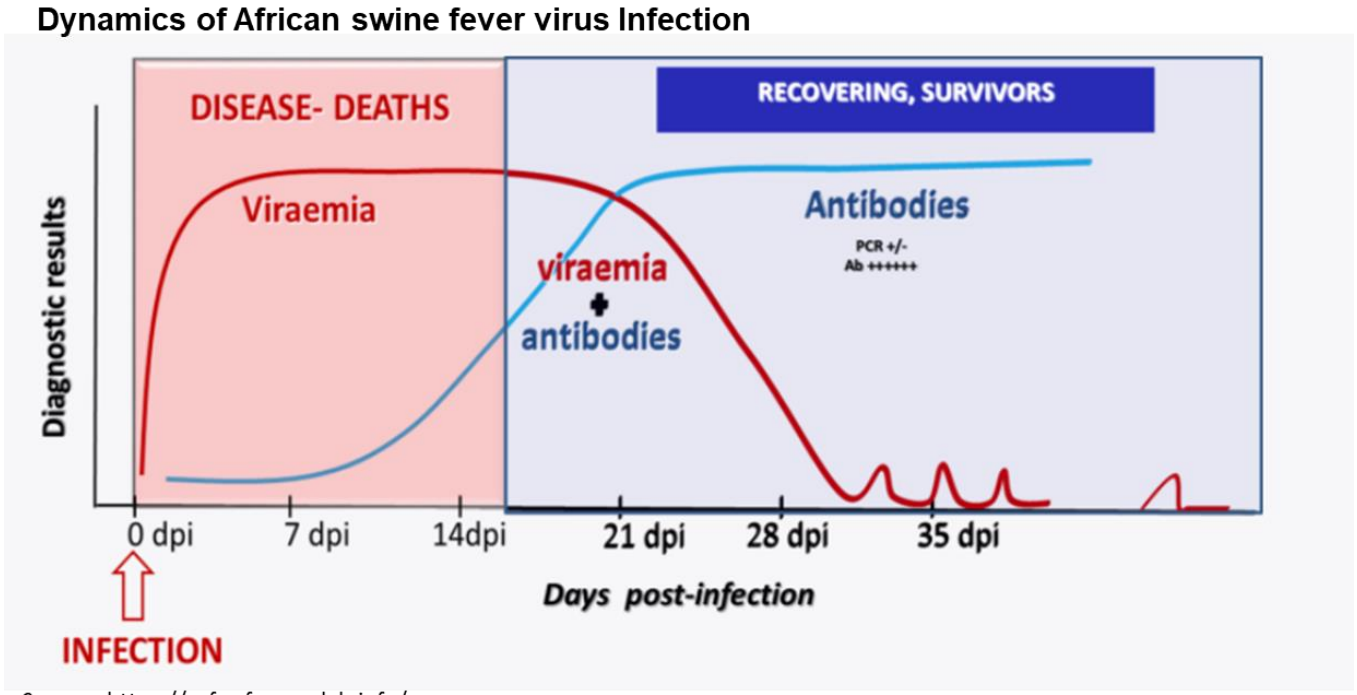
- Five genetically distinct strains of ASFV have been isolated from clinical cases of the disease outside the control area since 2016.
- This suggests that there have been at least 5 independent introductions of the virus.
- The exact sources of the strains remain unknown.

Phylogenetic Analysis



Serological Testing

- Serology is used for large scale surveillance due to its simplicity and comparatively low cost.
- ASFV antibodies appear 10-14 days after infection and persist for several years
- Since there is no vaccine against ASF, the presence of ASFV antibodies are always indicative infection
- In peracute and acute infections pigs often die before antibodies become detectable.



Source: https://asf-referencelab.info/

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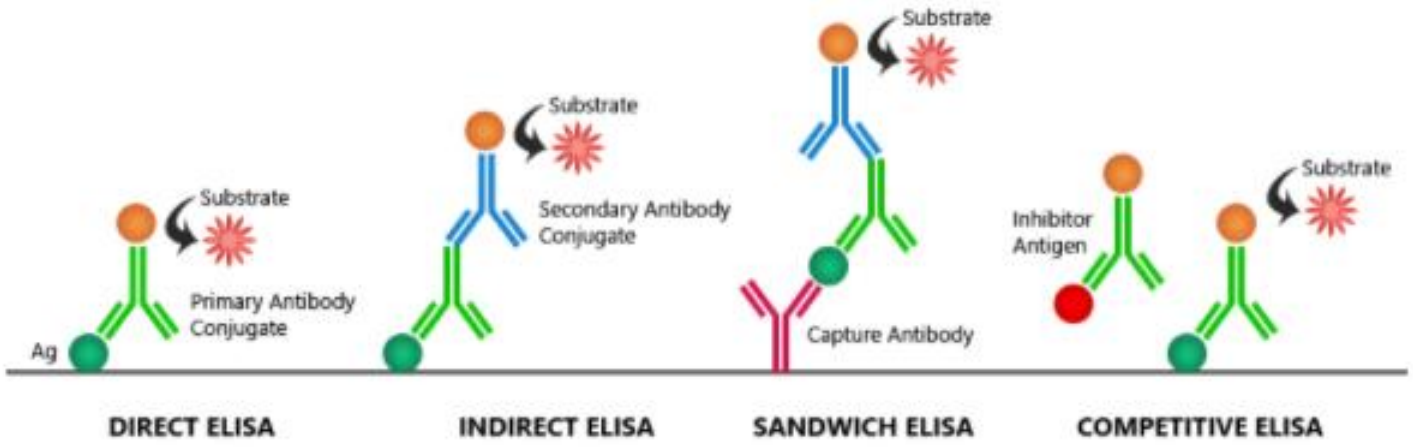
Enzyme Linked Immunosorbant Assays (ELISA)



Serological Testing

- Several commercial ELISA kits are available
- The Competitive ELISA can be used for multiple species, including warthogs

Sensitivity - 88%

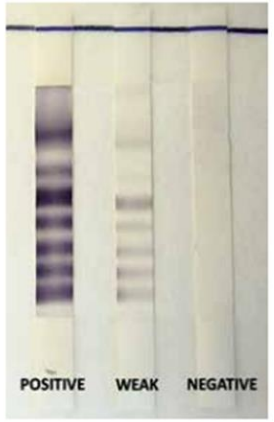
Specificity - 97%

- Positive serum should be confirmed using Immuno-blotting
- Pen-side tests base on lateral flow technology is available, but has not been fully validated for use in South Africa

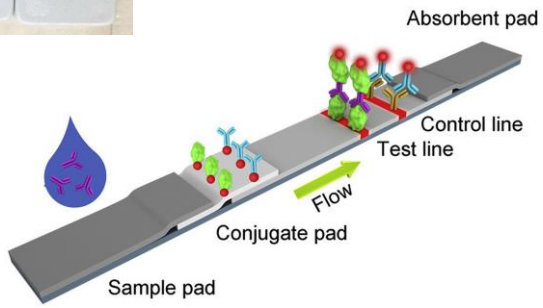
INgezim PPA COMPAC

https://www.youtube.com/watch?v=LbdRBf1imKo

ASF antibody detection by immunoblotting (IB)



Pen-side test for the detection of ASF antibodies



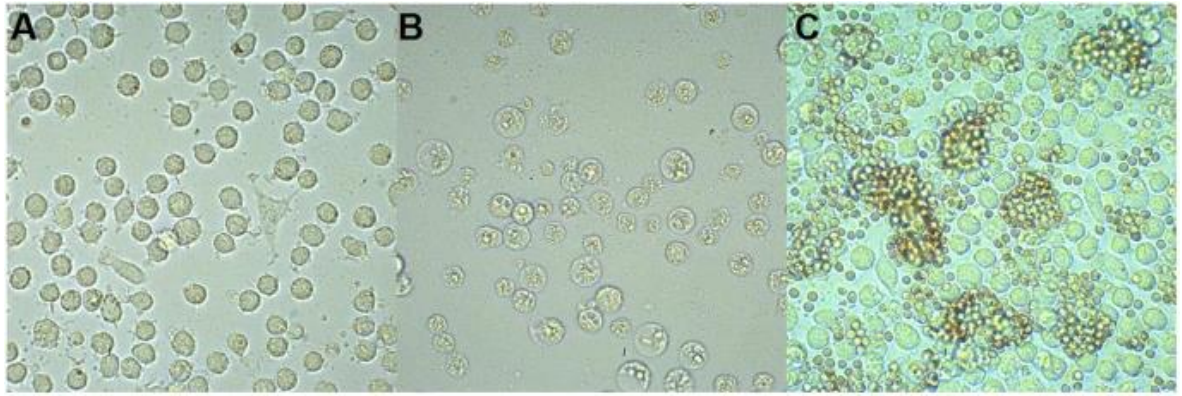
Source: Li et al., Talanta, Volume 219, 2020

Virus Isolation

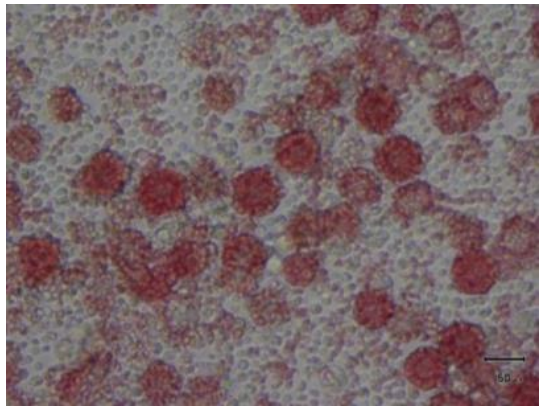
- ASFV is isolated using susceptible primary cell cultures of porcine origin
- ASFV produces a haemadsorption reaction (HAD) due to adsorption of red blood cell, forming “rosettes”
- Not all ASFV strains cause haemabsorbtion, requiring results of virus isolation to be confirmed using molecular tests

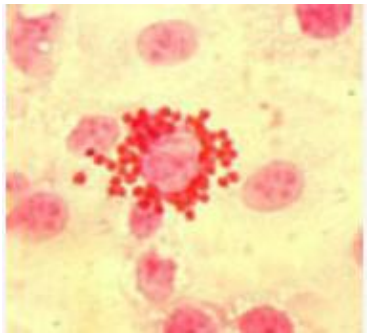
Haemadsorbtion

(A) Mock-infected cells (B) Cytopathic effect (C) Haemadsorption



Source: Mazur-Panasiuk et al. Sci Rep . 2019 Mar 14;9(1):4556.





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ASF Diagnostics at a glance

ASSAY FOR VIRUS DETECTION	TIME	SENSITIVITY	SPECIFICITY	SAMPLE TYPE	COST	COMMENTS
Polymerase Chain Reaction (PCR)*	5-6 hours	XXX	XX	Tissues, blood, ticks or cell cultures	$$	Most common method Susceptible to contamination Detects live or dead virus
Haemadsorption Test (HA)	7-21 days	XX	XXX	Porcine macrophage cells	$$$$	GOLD STANDARD Only used in a few reference laboratories
Direct Fluorescence Antibody test (FAT)	75 min	XXX (for early detection)	XXX	Cryostat sections. Impression smears. Cell culture of macerates	$$$	Recommended when PCR is unavailable or lack of experience Needs a fluorescent microscope Lack of sensitivity after the first week post-infection
Enzyme-Linked Immunosorbent Assay (ELISA)	3 hours	X (for early detection)	XX	Serum, macerates	$	Not routinely used Lack of sensitivity after the 1st week post-infection

ASSAY FOR ANTIBODY DETECTION	TIME	SENSITIVITY	SPECIFICITY	SAMPLE	COST	COMMENTS
Enzyme-Linked Immunosorbent Assay (ELISA)*	3 hours	X	X	Serum	$	Screening test In-house and commercial kits available
Immunoblotting	3 hours	X	X	Serum	$$$$	Confirmatory test No commercial kits
Indirect Fluorescent Antibody (IFA) test	4 hours	XXX	XX	Tissue exudates, serum or plasma	$$$	Confirmatory test No commercially available reagents Needs a fluorescent microscope

(*): most commonly used

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