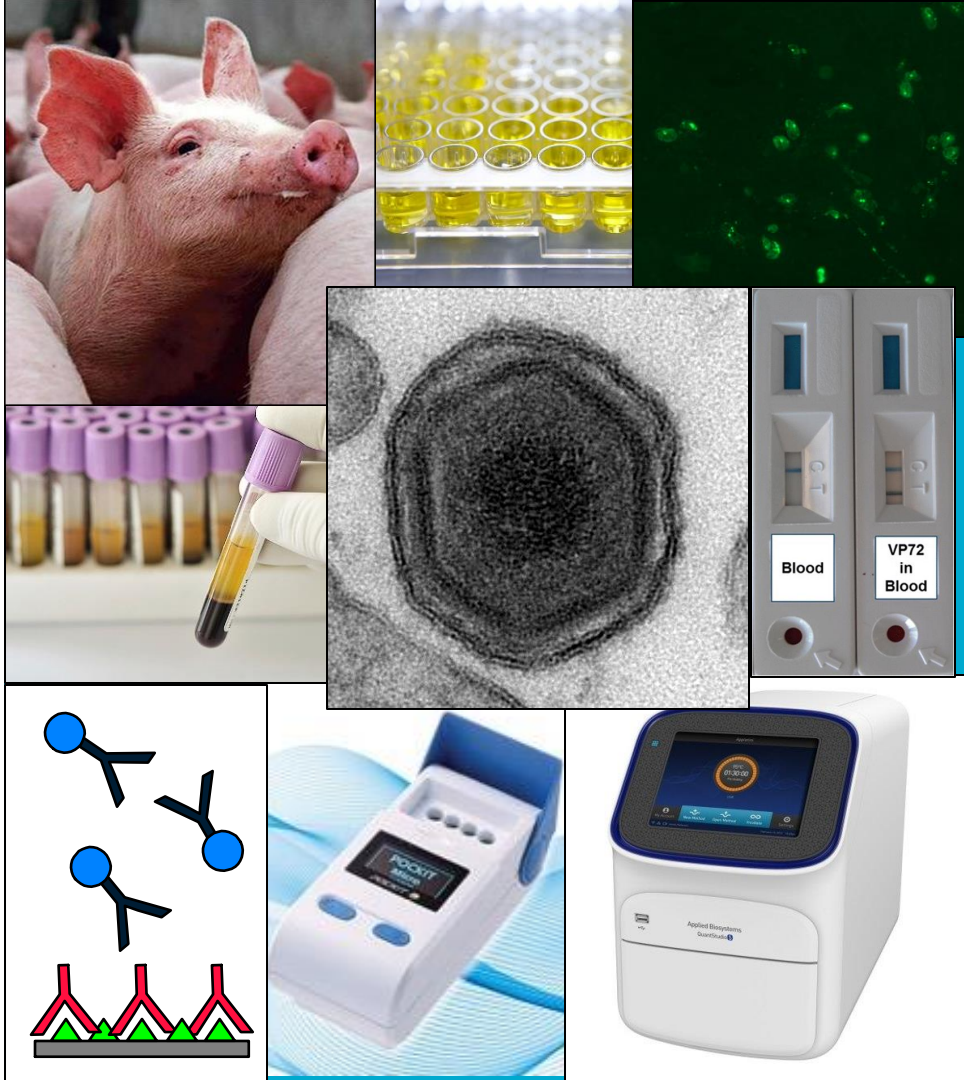
African swine fever laboratory diagnostics

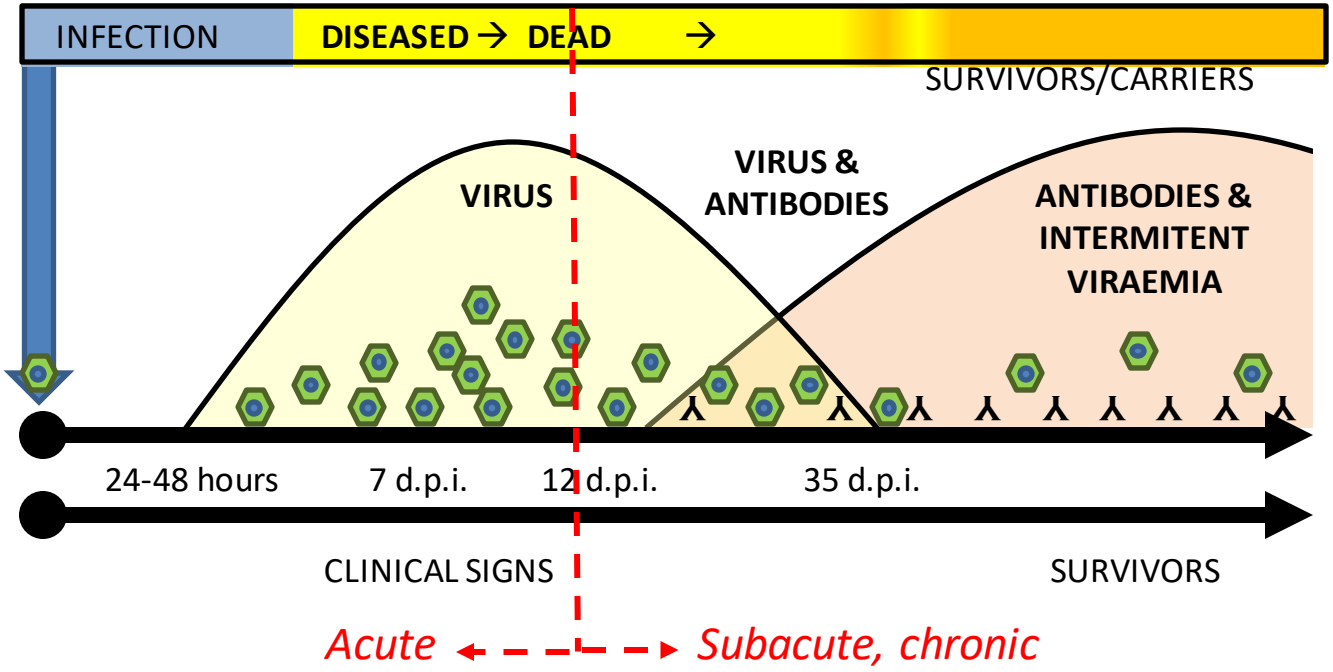
GF-TADs African swine fever (ASF) Coordination Virtual meeting

David Williams | 25th August 2021



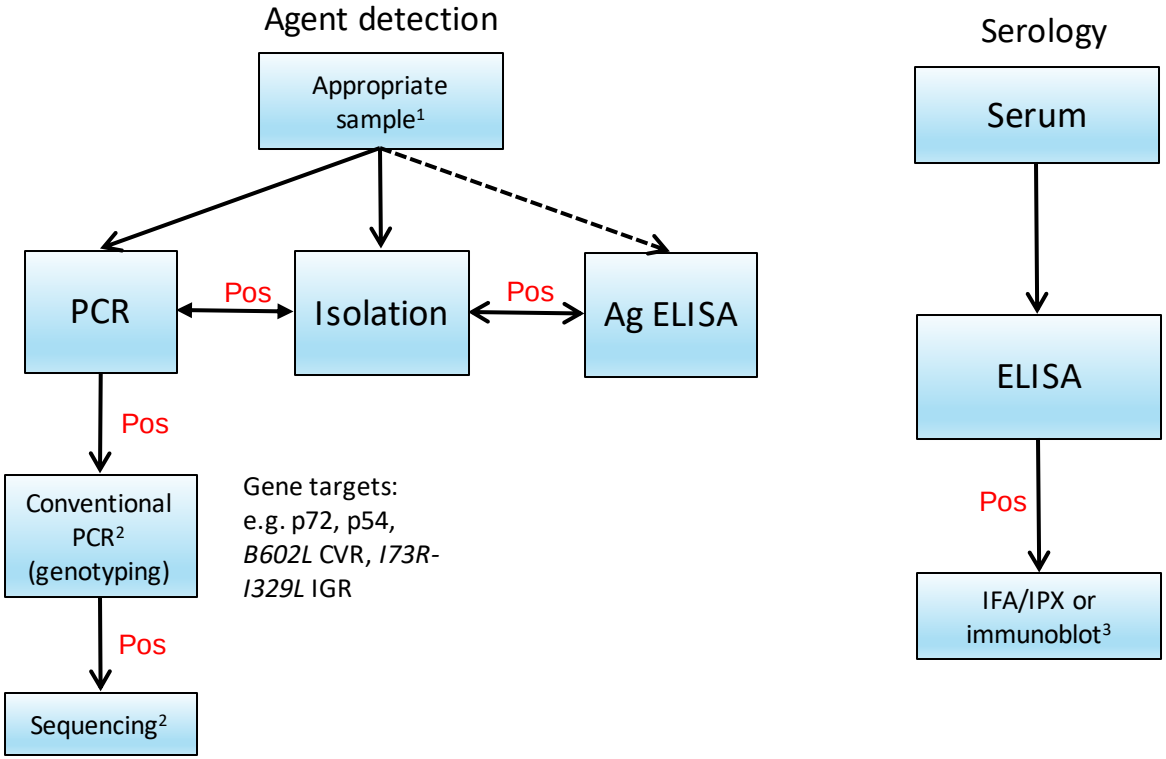
African swine fever diagnosis

- Since **no vaccine** available, rapid and early detection is essential to implement control measures
- ASF **cannot** be diagnosed based on clinical signs alone because of its similarity with other haemorrhagic diseases
  - e.g. CSF, PRRS, erysipelas
- Laboratory testing is therefore essential, using virus or antibody detection methods
- Several field diagnostic (pen-side) tests also available
- *Different forms of ASF now recognised in the region*



- Acute disease: **virus detection (PCR, antigen) is most useful**, serology of lower diagnostic value since most pigs die before antibody response (7-10 days)
- 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, lymph nodes, spleen, tonsils, kidneys
2. At start of outbreak/on selected isolates
3. For confirmation or clarification

PCR

- Detection of partial gene fragments of the ASFV genome (*B646L* gene encoding p72)
- **Rapid** (~4 hrs) and highly sensitive
- **Frontline choice for outbreak investigations** and **routine diagnostics**
- Can detect virus in absence of infectious particles or when at low levels

  →Decomposed tissues, pork products

  →Low/moderate virulence strains

Assay	Target	Format	OIE	Reference
Aguerro	VP72	Conventional	Y	Aguerro et al. 2003. J. Clin. Micro. 41:4431
King	VP72	Realtime	Y	King et al. 2003. J. Virol. Methods, 107:53
UPL	VP72	Realtime	Y	Fernández-Pinero et al. 2013. Trans. Emerg. Dis. 60:48
USDA	VP72	Realtime	N	Zsak et al. 2005. J. Clin. Micro. 43: 112
McKillen	9GL	Realtime	N	McKillen et al. 2010. J. Virol Methods. 168:141
Tignon	VP72	Realtime	N	Tignon et al. 2011. J. Virol. Methods. 178:161
Haines*	VP72	Realtime	N	Haines et al. 2013. PLoS ONE. 8: e71019
Luo	VP72	Conventional	N	Luo et al. 2017. Arch. Virol. 162:191
Ingenasa	VP72	Realtime	N	Based on UPL; INgene q PPA
IDEXX	?	Realtime	N	RealPCR ASFV DNA Mix
ID.Vet	?	Realtime	N	ID Gene® African Swine Fever Duplex
Tetracore	VP72	Realtime	N	Based on USDA assay
AB	VP72	Realtime	N	VetMAX ASF kit
Indical	VP72	Realtime	N	*Virotype*® ASFV PCR (based on Haines assay)

**ASFV/CSFV duplex*

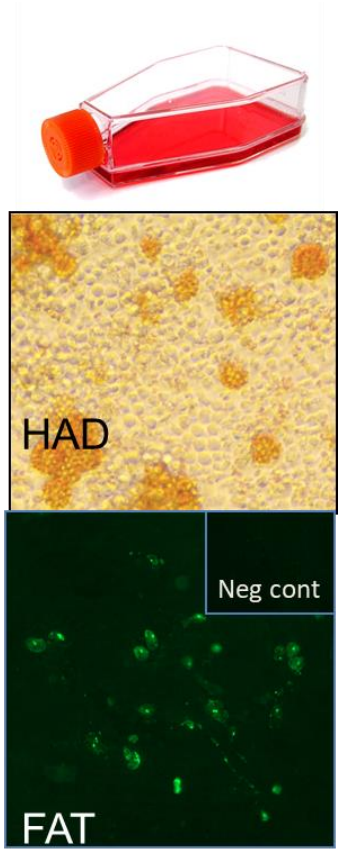
Antigen detection

- **Double sandwich ELISAs**
  - Inexpensive and useful for large scale testing
  - Do not require specialised equipment
  - Commercial ELISA available (Ingezim PPA DAS K2)
    - Diagnostic Se relatively low ~77% (Gallardo et al., 2015)

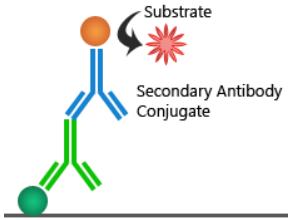
- *Because of low sensitivity, recommended as a 'herd test'*
- Can be used for primary diagnosis, if no PCR capacity

Virus isolation

- Inoculation of specimen onto primary porcine cells
  - Bone marrow or alveolar macrophages
  - Some cell lines (e.g. MA-104), but less sensitive
- Virus detection by:
  - Haemadsorption assay, immuno-detection, PCR or Ag ELISA
- Expensive to maintain capability
  - Specialised facilities (BSL3), equipment and expertise
- Recommended as a reference test when ASFV has been detected by other methods
  - Especially for primary outbreaks or index cases



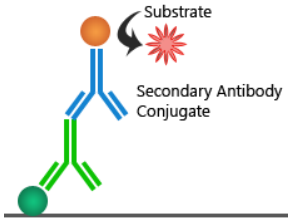
Serology: ELISA



- Most commonly used test for antibody detection
  - Recommended for screening
- OIE-recommended 'in-house' ELISA (Pastor et al 1990), using solublised infected cell extract Ag
- Commercial ELISAs available in indirect or competitive formats:

Kit/test	Format	Antigen	Sample type
ID.vet ID Screen	Competitive	P30	Serum, plasma
ID.vet ID Screen	Indirect	P30, P62, P72	Serum, plasma, meat juice, blood/filter paper
Svanovir ASFV-Ab	Indirect	P30	Serum, plasma
Ingenasa INgezim PPA Compac 1.1PPA.K.3	Competitive	P72	Serum
Ingenasa INgezim ASF ASFV-R	Indirect	cp312, p30	Serum, spleen exudate, blood/filter paper

ELISA



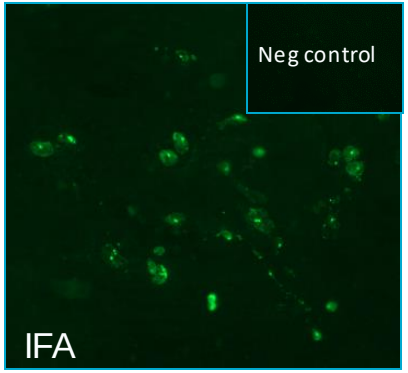
- **Advantages:**
  - Rapid testing and interpretation
  - High throughput, automation
- **Disadvantages**
  - Less sensitive than IPX/IFA, may under-represent seroprevalence in surveillance studies
  - Prone to reduced specificity when poor quality samples used

    → *Confirmatory testing important (e.g. at Ref lab)*

Other serological tests

Immunofluorescence/Immunoperoxidase

- Recommended for confirmatory testing
- Fixed, infected monolayers
  - Glass slides or 96-well plate format
- **Advantages:**
  - Highly sensitivity, can detect antibodies early in infection
  - Can be adapted for testing large numbers of samples (96-well format)
- **Disadvantages:**
  - Labour-intensive
  - Requires virus culture capability at BSL3 (or access to fixed slides/plates)
  - Requires microscopic examination of each test
  - Variation in operator interpretation may occur



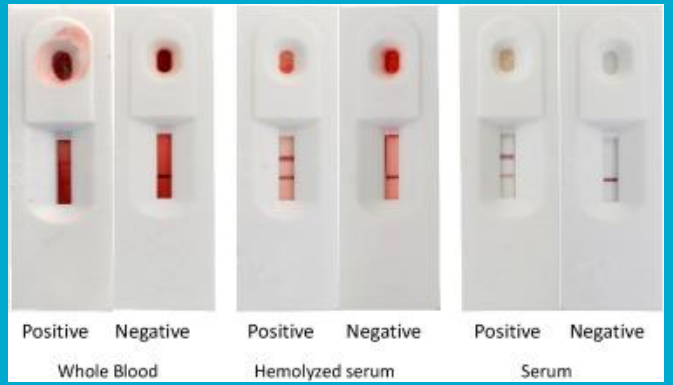
Source: ACDP

Field tests: Antigen detection

- Early detection for rapid response at or near outbreak
- Fresh EDTA blood (serum, plasma)
- Several commercial options
  - Lateral flow or dip stick
  - Rapid: 10-25 mins
- DSe/Sp:
  - Ingenasa: ~68%/99%* (Sastre et al. 2016)
  - Shenzhen: ~65%/76%* (Matsumoto et al. 2020)
  - PenCheck: *No peer reviewed report*
  - Bionote: *No peer reviewed report*

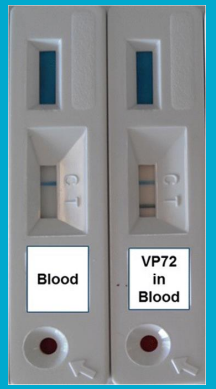
**Diagnostic Sensitivity/Specificity*

Shenzhen LB

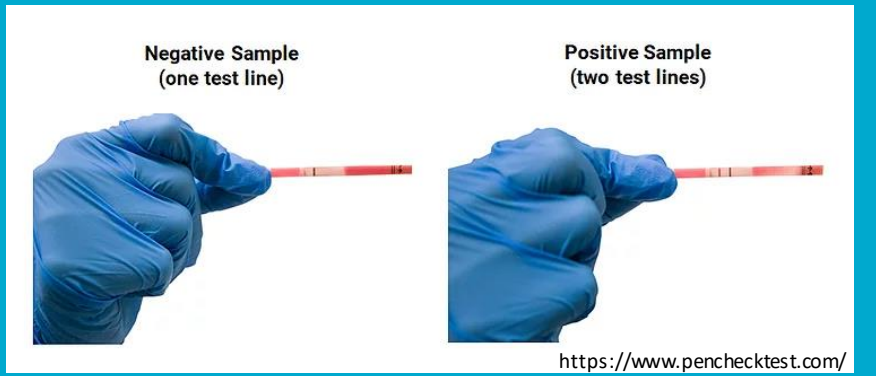


Matsumoto et al. 2020 J. Virol. Methods

Ingenasa



Sastre et al. 2016 BMC Vet Res.



PenCheck

Field tests: Molecular

- PCR or isothermal methods
- Can be used in the field or in small labs

Example: POCKIT PCR

- Analytical and diagnostic sensitivity and specificity comparable to real-time PCR
  - Se/Sp ~100% (FAO)
- Requires DNA extraction
- *~2 hours*

Source: Dr. Ken Inui, FAO

Field tests: Molecular

Example: LAMP

- No extraction required for whole blood (1:10) or serum
  - Tissues, pork need to be extracted
  - *~30 mins*
- Colorimetric or fluorescence
- Analytical sensitivity lower than real time PCR
  - ~300 gene copies vs ~10-20 copies
- Diagnostic Se and Sp comparable to real-time PCR, up to 100%

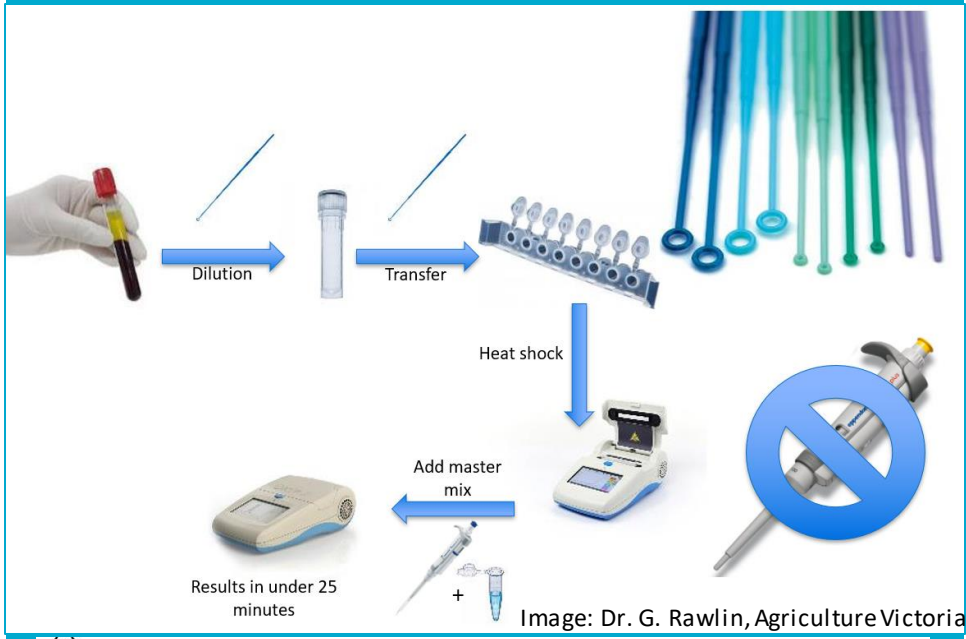
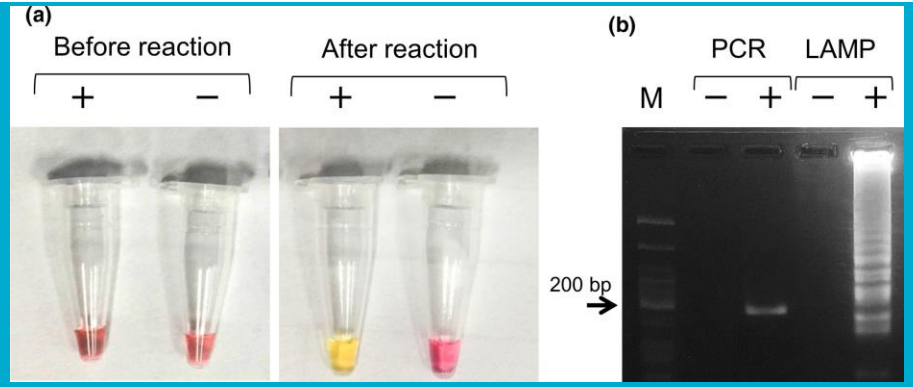


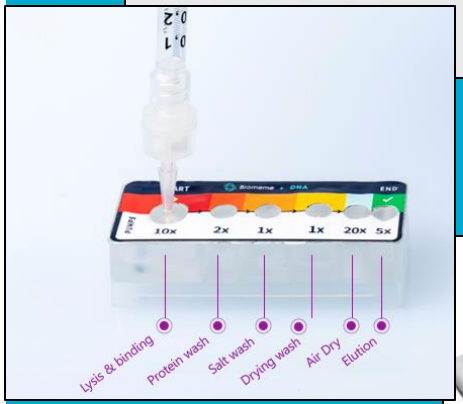
Image: Dr. G. Rawlin, Agriculture Victoria



Tran *et al.* 2020. Trans. Emerg. Dis.

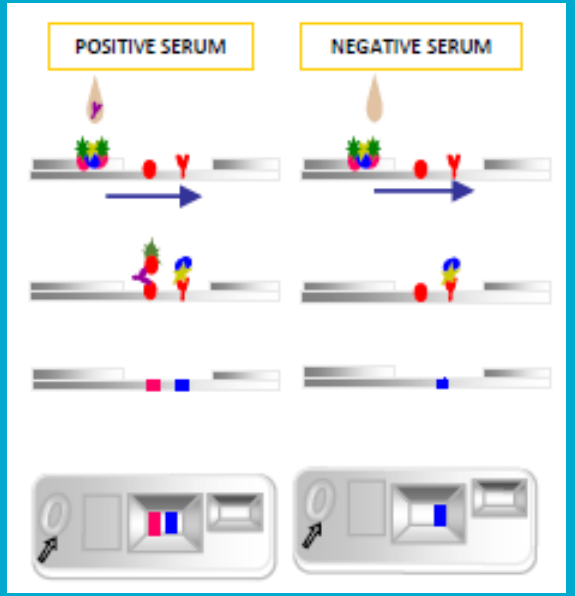
Field tests: Molecular

- Other commercial PCR platforms:
  - Biomeme
  - Indical IndiField (+PCR kit)
  - Tetracore (+PCR kit)
  - Genesig (+PCR kit)



Field tests: Antibody detection

- Lateral flow devices
- Ingenasa ASF CROM Ag one-step immunochromatographic test
  - Comparable to ELISA
    - Se 99%, Sp 100% (Perez et al. 2011)
  - Evaluated for field use with wild boars (Cappai et al. 2017)
    - Se (81.8%), Sp (95.9%)
- Excelsior Biosystem Sentinel® ASF Virus Antibody Rapid Test
- Bionote ASFV Ab Test Kit



Source: Ingenasa product insert

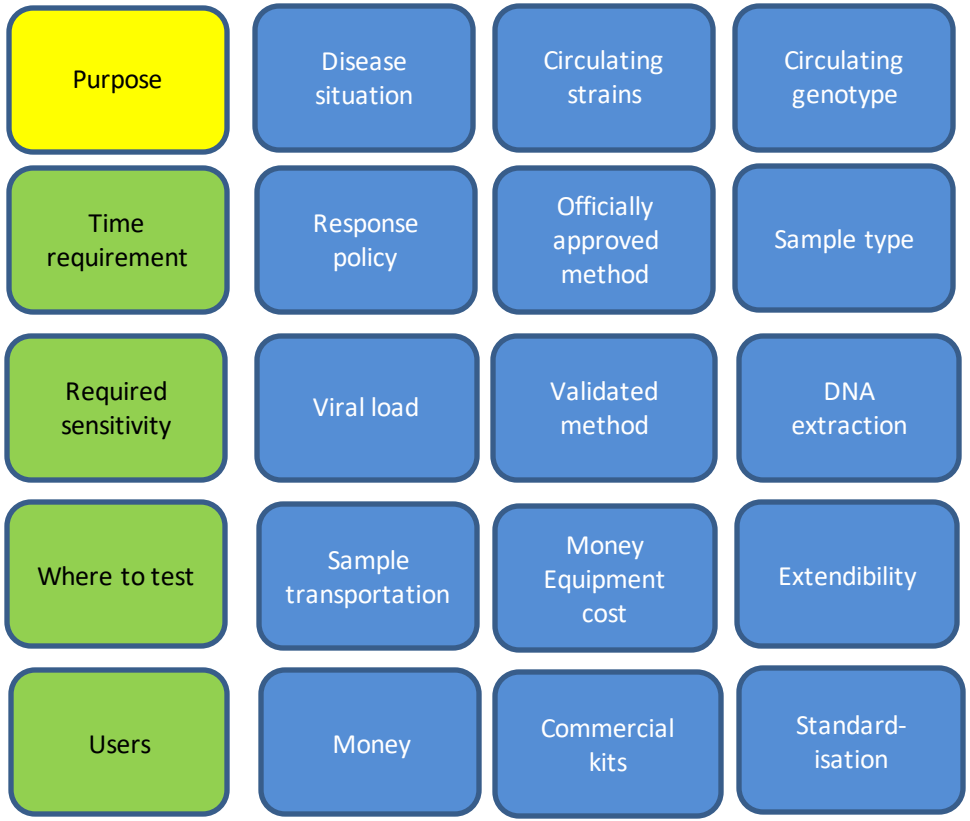
Tools of ASF virus testing and Factors affecting choices

Tools for virus/antibody detection

1. **DNA detection**
   a. Conventional PCR
   b. Real-time PCR
   c. Point-of-care PCR, LAMP

2. **Antigen detection**
   a. ELISA
   b. Rapid test

3. **Antibody detection**
   a. ELISA
   b. Rapid test
   c. IFA/IPX/IB



Source: Dr. Ken Inui, FAO

Selection of virus detection tests in different scenarios

Purpose		Who/Where	Sample	Requirements		Virus detection methods				
						Ag detection		DNA detection		
				Time	Sensitivity	Rapid test	Ag ELISA	Conventional PCR	POC PCR (LAMP, etc)	Real-time PCR (or equivalent)
						Sensitity low	Sensitivity medium		Sensitivity High	
1. Outbreak investigation										
a	Diagnosis of ASF suspected cases	Field Vets	WB	<2hrs	Low	X			X	
b	Diagnosis of ASF suspected cases	Provincial lab	WB	<24hrs	Low	X	X	X	X	X
c	Diagnosis of ASF suspected cases	Government lab	WB	<24hrs	Low	X	X	X	X	X
2. Monitoring/surveillance of virus circulation										
a	Market	Government lab	Meat	<24hrs	High				X	X
b	Slaughterhouse	Government lab	WB	<2hrs	High				X	X
e	Farm	Government lab	WB	<24hrs	High				X	X
3. Movement control										
a	Quarantine stations	Quarantine	WB/Swab	<24hrs	High				X	X
b	Check points between regions/ provinces	Check-point	WB/Swab	<2hrs	High				X	X
c	Health certificate for pig movement	Government lab	WB	<24hrs	High				X	X
4. Biosecurity check/routine testing at large farm										
a	Routine testing of dead pigs	Producer	WB/Swab	<2hrs	Low	X	X	X	X	X
b	Routine testing of sick pigs	Producer	WB/Swab	<2hrs	Medium	X	X	X	X	X
c	Quarantine for in-coming replacement pigs	Producer	WB	<24hrs	High				X	X
d	Check truck, feed, semen, equipment	Producer	Swabs	<2hrs	High				X	X

WB, whole blood

Source: Dr. Ken Inui, FAO

Selection of antibody detection tests in different scenarios

Purpose		Who/Where	Sample	Requirements		Antibody detection methods		
				Time	Sensitivity	Rapid test	ELISA	IPX/IFA assay
1. Outbreak investigation (especially for low virulent/ chronic strains)								
a	Diagnosis of ASF suspected cases	Field Vets	Serum	<2hrs	Low	X		
b	Diagnosis of ASF suspected cases	Provincial lab	Serum	<24hrs	Low		X	
c	Diagnosis of ASF suspected cases	Government lab	Serum	<24hrs	Low		X	X
2. Monitoring/surveillance of virus circulation								
a	Market	Government lab	Meat juice	<48hrs	High		X	X
b	Slaughterhouse	Government lab	Serum	<2hrs	High	X		
e	Farm	Government lab	Serum	<24hrs	High		X	X
3. Movement Control								
a	Quarantine stations	Quarantine staff	Oral fluid	2-24hrs	High	X	X	
b	Check points between regions/provinces	Check-point staff	Oral fluid	<2hrs	High	X		
c	Health certificate for pig movement	Government lab	Serum	<24hrs	High		X	X

Source: Dr. Ken Inui, FAO

Comparison of PoC vs lab methods for virus detection

	Antigen detection	DNA detection		
	Point of care (POC) Test			Laboratory
Test	**Rapid test (lateral flow device)**	**Isothermal (LAMP, Pockit, etc)**	**Mobile real-time PCR**	**Lab-based real-time PCR**
Intended Use	Screening test	POC detection with high sensitivity and specificity		Confirmatory test
Specimen Type(s)	Blood (serum, plasma)	Blood, serum (tissues)	Blood, serum (tissues)	All specimens
Sensitivity	Low to moderate	High	High	High
Specificity	High	High	High	High
Training	No	Yes	Yes	Yes
Turnaround Time	15 to 30 min	30 to 120 min	60 to 120 min	60 to 120 min plus sample transportation time
Cost/test	$US 4 to 10	$6 to 25 including DNA extration	$6 to 15 including DNA extration	$6 to 15 including DNA extration
Cost of equipment	None	$1,000 to 15,000	$7,000 to 15,000	$30,000+
Advantages	Quick (early detection at POC)	High sensitivity and specificity	High sensitivity and specificity	High sensitivity and specificity
	Easy (anyone can perform)	POC detection	POC detection	Official confirmatory test
	Cheap			High throughput
				Validated assays and commercial kits
Disadvantages	Sensitivity low to moderate, but good enough for very sick and dead animals	Relatively high equipment cost	Relatively high equipment cost	High equipment cost
Use	Outbreak investigation	Outbreak investigation	Outbreak investigation	Outbreak investigation
	Routine test for sick pigs	Routine test for sick and mortality	Routine test for sick and mortality	Routine test for sick and mortality
		Quarantine	Quarantine	Quarantine
		Biosecurity check	Biosecurity check	Biosecurity check
				Movement control
				Surveillance
Comments	Needs evaluation of new products	Many products coming up. Major tool in the future?		Gold standard
		Suitable for small labs. Automated system available		

Source: Dr. Ken Inui, FAO

Diagnostic tests – general considerations

- Several commercial and in-house options available for PCR, antigen and antibody detection
  - Laboratory-based
  - Field-based (PoC)
- *Different diagnostic tools can be used for different scenarios and for different forms of ASF*
  - *Selection based various factors*
  - *Acute vs chronic ASF*
  - *Endemic vs outbreak response*

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