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

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Cover photos:

On-site detection of pathogens in Vietnam / Ken Inui

Pius Clement of the National Agriculture Quarantine & Inspection Authority (NAQIA), conducting an inspection in Papua New Guinea / David Williams

OIE Reference Laboratory Network
African Swine Fever

Disclaimer:
This document summarizes the current knowledge of the OIE ASF Reference Laboratory Network on commercially available Point of Care (PoC) tests. The mention of specific companies or products of manufacturers, whether or not these have been patented, does not imply that these have been endorsed or recommended by the OIE in preference to others of a similar nature that are not mentioned. All commercial kits should be validated according to the OIE international standards. All commercial kits included in the OIE Register are certified by the OIE as validated and fit for purpose. The PoC tests included in this document are not in the OIE Register nor are they described in the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. The OIE Register can be consulted at: *https://www.oie.int/en/what-we-offer/veterinary-products/#ui-id-5*

INTRODUCTION

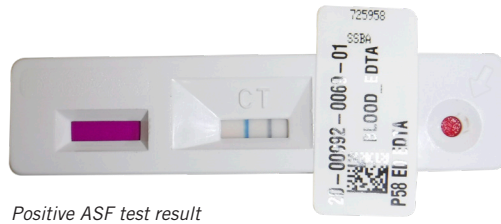
On-site testing with portable PCR in Vietnam / Ken Inui

African swine fever (ASF) cannot be differentiated from other febrile haemorrhagic syndromes or bacterial septicaemias of pigs by either clinical or post-mortem examination. Laboratory tests are, therefore, essential for the diagnosis of ASF and are key to the success of ASF surveillance activities.

Chapter 3.9.1. of the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Terrestrial Manual)* describes the recognised international standards for ASF diagnosis. However, in certain circumstances, the timely submission, processing and testing of samples using the diagnostic tests described in the *Terrestrial Manual* are not feasible.

The ability to test for ASF at the point of disease allows for rapid response to outbreaks and control of the spread of disease in endemic situations.

Despite not being included in the Register of Diagnostic Kits certified by the OIE as validated as fit for purpose, there are several diagnostic platforms, also known as penside or point-of-need/ point-of-care testing (PoC tests), that are available commercially for field testing. These include basic rapid test kits for detecting antigens or antibodies using lateral flow devices that are simple to use, require minimal training and can provide a result within approximately 20 minutes.

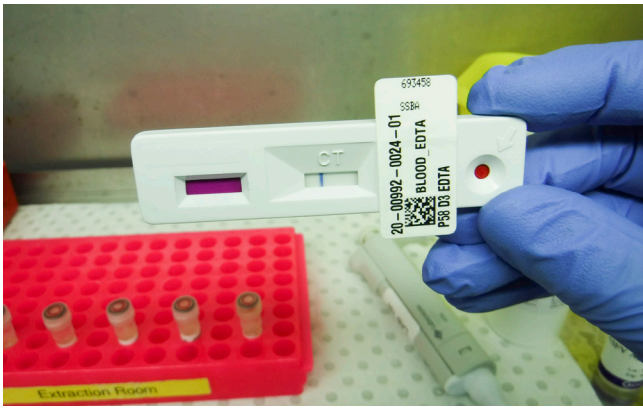


Positive ASF test result

Rapid antibody tests generally have comparable levels of sensitivity and specificity to laboratory enzyme-linked immunosorbent assays (ELISAs), and lower sensitivity compared with reference tests such as the immunoperoxidase monolayer assay. These tests can be used to detect antibodies in pigs that have survived infection or have survived long enough to seroconvert.

Rapid antigen tests are typically less sensitive than molecular techniques for virus detection, but some can have comparable levels of specificity. Antigen tests are recommended for use on symptomatic and terminally ill pigs that have high levels of viraemia, rather than on pigs in the early stages of clinical infection that may not have high enough viraemia to allow detection. It is recommended that samples from more than one sick pig are tested to increase the chances of detecting infection.

There are also several molecular platforms now available that allow very sensitive ASF virus DNA detection in infected pigs, even at the early stages of disease. These tests can also be used to detect contaminated carcasses, and pork and environmental samples at the point-of-need (e.g. abattoir, airport or wild boar/feral pig habitats). However, these platforms are technically more complex than rapid antibody or antigen tests and require a much higher level of training and competency for accurate testing. Molecular field tests also require expensive equipment for amplification and, in many cases, for extracting viral DNA.



Negative ASF test result

The choice of which method to use can be influenced by many factors, including costs, ease of use and training requirements. Simple rapid tests may be appropriate for certain situations, such as resource-poor settings, while 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 the specific setting where the test will be used (e.g. farm, abattoir, meat market, port of entry) and available resources.

This document aims to summarise the current knowledge of the OIE ASF Reference Laboratory Network on commercially available PoC tests, including a range of technical details, cost, as well as advantages and disadvantages of each. The tests were selected based on peer-review publications reporting evaluation of the tests or platforms or based on independent evaluation at the laboratories of the authors. It is important to note that PoC tests are a very useful adjunct to, but not a replacement for, laboratory testing in ASF disease control programmes. The results obtained using the PoC tests described in this document would need to be confirmed by a laboratory using the diagnostic tests described in Chapter 3.9.1. of the *Terrestrial Manual*.

Table 1. Comparison of four major test platforms for ASF 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)	Whole blood, serum, plasma*	Whole blood, serum, plasma, tissues, swabs*	Whole blood, serum, plasma, tissues, swabs*	Whole blood, serum, plasma, tissues, swabs, pork, environmental samples
Sensitivity	Low to moderate	High	High	High
Specificity	High	High	High	High
Training	No (low)	Yes	Yes	Yes
Testing time	15 to 30 min	40 to 120 min	60 to 120 min	60 to 120 min plus sample transportation time
Cost/test (US$)	2.50 to 14.00	4.00 to 23.00, including DNA extraction	5.00 to 15.00, including DNA extraction	6.00 to 15.00, including DNA extraction
Cost of equipment (US$)	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 high enough for very sick and dying animals	Relatively high equipment cost	Relatively high equipment cost	High equipment cost
				Specialised laboratory requirements
				Highly trained staff
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.		

** Some tests are designed for use with certain sample types; limited evaluation of sample types for some platforms has been reported.*

Table 2. Comparison of four major PoC test methods for rapid ASF virus antigen detection

Test	Ingenasa	Bionote	PenCheck™	Shenzhen Lvshiyuan Biotechnology Co.
Catalogue no.	INgezim ASF CROM Ag (11.ASFV.K.42)	Anigen ASFV Ag Rapid Test (RG1407DD)	Rapid Screening Test for ASFV (PC-888)	SLB ASF Antigen Detection RDT
Website	*ingenasa.eurofins-technologies.com/home/*	*www.bionote.co.kr*	*www.pencheckte st.com/*	*lsybt.com/En*
Specimen type(s)	Whole blood	Whole blood, serum, plasma	Whole blood	Whole blood
Format	Lateral flow	Lateral flow	Dipstick	Lateral flow
Level of assessment	Peer-reviewed published journal article			Peer-reviewed published journal article
	Independent assessment at Reference Laboratories	Independent assessment at Reference Laboratories	Independent assessment at Reference Laboratories	Independent laboratory assessment
Sensitivity	Low to moderate (~68%)	Low to moderate*	Low*	Low to moderate (~65%)
Specificity	High (98%)	Moderate*	Moderate to high*	Moderate (~76%)
Training	Low	Low	Low	Low
Testing Time	15 min	20 min	25–30 min	15–20 min
Cost/test (US$)	5.80 to 10.45 (depending on pack size)	13.90	2.50	3.50
Cost of equipment	None	None	None	None

Table 2 cont'd. Comparison of four major PoC test methods for rapid ASF virus antigen detection

Test	Ingenasa	Bionote	PenCheck™	Shenzhen Lvshiyuan Biotechnology Co.
Advantages	Rapid (early detection at PoC)	Rapid (early detection at PoC)	Rapid (early detection at PoC)	Rapid (early detection at PoC)
	Easy (anyone can perform)	Easy (anyone can perform)	Minimal training (e.g. pipette use)	Easy (anyone can perform)
	Inexpensive	Inexpensive	Inexpensive	Inexpensive
	No equipment costs	No equipment costs	Minimal equipment required (pipette and tips for aliquotting test reagent)	No equipment costs
	High specificity		Moderate to high specificity	
Disadvantages	Sensitivity low to moderate, but high enough for testing very sick and dying animals	Sensitivity low to moderate, but high enough for testing very sick and dying animals; moderate specificity (--> false positives)	Low sensitivity	Sensitivity low to moderate, but high enough for testing very sick and dying animals; moderate specificity (--> false positives)
Comments	Evaluated at CISA-INIA, ACDP, NVLD and Pirbright; analytical sensitivity between 6-7 $\log_{10}$ $TCID_{50}$ ASFV in spiked blood and 7.75 $\log_{10}$ $TCID_{50}$ in blood from experimentally infected pigs (Pirbright)	Evaluated at ACDP using blood from experimentally infected pigs: 68% of PCR* positive samples were positive; 90% of PCR negative samples were negative	Evaluated at ACDP using blood from experimentally infected pigs: 27% of PCR* positive samples were positive; 92% of PCR negative samples were negative	Peer-reviewed published journal article
References	Sastre *et al.* (2016a)	Peer-reviewed publication not yet available	Peer-reviewed publication not yet available	Matsumoto *et al.* (2020)

**In-house ASF PCR described by Zsak* et al. *(2005) used for ACDP for evaluation*

Table 3. Comparison of three major PoC test methods for rapid ASF virus antibody detection

Test	Ingenasa (ASFV/CSFV duplex)	Ingenasa (ASFV)	Global Dx
Catalogue no.	INGEZIM ASFV-CSFV CROM Ab (11.SFV.K41)	INGEZIM PPA CROM (11.PPA. K41/25)	GDX70-2 Herdscreen® ASF Antibody
Website	*ingenasa.eurofins-technologies.com/home*	*ingenasa.eurofins-technologies.com/home*	*globaldx.com*
Specimen type(s)	Whole blood, serum	Whole blood, serum, plasma	Whole blood, serum, plasma
Format	Lateral flow	Lateral flow	Lateral flow
Level of assessment	Peer-reviewed published journal article	Peer-reviewed published journal article	
	Independent assessment at reference laboratories	Independent assessment at reference laboratories	Independent assessment at reference laboratories
Sensitivity	Moderate to high (CSFV-92%/ ASFV-87%)	Moderate to high (82% sensitivity with respect to the immunoperoxidase monolayer assay [IPMA] in wild boar; 99% correspondence to ELISA)	Moderate to high analytical Ss. (Correspondence with IPMA is 86.2%. Equivalent or higher sensitivity than the commercial ELISAs)
Specificity	High (98.4%-CSFV/ASFV-100%)	High (99.9% correspondence with ELISAs. 96% specificity respect IPMA [wildboar])	High (100% correspondence with reference technique IPMA)
Training	Low	Low	Low
Testing Time	15 to 30 min	15 to 30 min	15 to 30 min
Cost/test (US$)	16.38	5.43 to (depending on pack size)	4.80
Cost of equipment	None	None	None

Table 3 cont'd. Comparison of three major PoC test methods for rapid ASF virus antibody detection

Test	Ingenasa (ASFV/CSFV duplex)	Ingenasa (ASFV)	Global Dx
Advantages	Rapid (early detection at PoC)	Rapid (early detection at PoC)	Rapid (early detection at PoC)
	Easy (anyone can perform)	Easy (anyone can perform)	Easy (anyone can perform)
	Inexpensive	Inexpensive	Inexpensive
	No equipment costs	No equipment costs	No equipment costs
	Differential diagnosis of CSFV-ASFV		
Disadvantages	Moderate diagnostic sensitivity for ASFV antibody detection. It is recomended to use in parallel with the Ag LFA	Moderate diagnostic sensitivity for ASFV antibody detection. It is recomended to use in parallel with the Ag LFA	Requires further field validation
Comments	Evaluated and validated at CISA-INIA, and NRLs for ASFV	Evaluated and validated at CISA-INIA, and NRLs for ASFV. Of 17 IgM or IgG positive samples tested at Pirbright, 15 were positive by LFD (8 weak positive)	Evaluated and validated at CISA-INIA and Pirbright
References	Sastre *et al*. (2016b)	Cappai *et al*. (2017)	Peer-reviewed publication not yet available

Workers in a backyard pig farm in the Philippines draw samples for testing / Bureau of Animal Industry (BAI), Philippines

Table 4. Comparison of Point-of-Care PCR systems for rapid ASF virus DNA detection

System		Test methods					
		iiPCR	iiPCR+DNA extraction (fully automatic)	LAMP	qPCR	qPCR	qPCR
Manufacturer		GeneReach	GeneReach	OptiGene	Tetracore	Indical	Genesig
Website		www.genereach.com	www.genereach.com	www.optigene.co.uk	tetracore.com	www.indical.com	www.genesig.com/home
PoC instrument	Instrument	POCKIT Micro Duo Nucleic Acid Analyzer	POCKIT Central	Genie III	T-CORE8	Indifield portable PCR system	Genesig q16 qPCR instrument
	Catalogue code	apmd	apcc	Gen3-01	T-CORE8	IF-IN6010093	Z-genesig-q16
	No. of wells	4	8	8	8 (independent)	9	16
	Testing time	45 min	45 min	30 min	60 min	30-60 min	60 min
	Power source	Battery	100-240 V	Battery /100-240 V	Battery /100-240 V	Battery /100-240 V	100-240 V
	Detection colors	2	2	2	up to 6	2	2
	PCR tube	Included in reagent kit	Included in reagent kit	standard 200 ul	Manufacturer's	standard 200 ul	Manufacturer's
	Weight (kg)	0.43	21	1.75	4.5	1.2	2
	Cost (US$)	3,000	30,000	18,000	20,000	9,000	9,000
Nucleic acid test reagents	Manufacturer's ASF kit	POCKIT ASFV reagent set; lyophilised	ASFV pre-mix cartridge; lyophilised	No	ASF w/IC (96 reactions) Wet assay	Virotype ASFV PCR Kit /IndiField ASFV PCR; lyophilised	Z-Path-ASFV
	In-house*	No	No	Yes	Yes	Yes	No
	Commercial kits**	No	No	Yes	Yes	Yes	No
	Cost (US$) / test	8	15 (includes DNA extraction)	4 to 23	5 to 15	5 to 15	5 to 15
DNA extraction	Instrument	taco Mini (8 wells); battery-operated; US$ 6000	Included in PCR	No	No	No	No
	Manufacturer's kit	Pre-loaded taco nucleic acid extraction kits (atc-pd/rna)	Included in PCR	No	(MagMAX™-96 Total RNA Isolation Kit)	M1 sample prep cartridge kit	Genesig easy DNA/RNA extraction kit
	Sample type	Whole blood, serum, tissues	Whole blood, serum, tissues	Serum, swabs***	Whole blood, tissues	Whole blood, serum, plasma, tissue, swabs	Various specimen types
	Time	30 min	40 min	95C for 2 min	30 min	2 min	60 min
	Cost (US$) / test	5.00	0.00	0.00	5.00 to 10.00	5.00 to 10.00	5.00 to 10.00

Table 4 cont'd. Comparison of Point-of-Care PCR systems for rapid ASF virus DNA detection

System		Test methods					
		iiPCR	iiPCR+DNA extraction (fully automatic)	LAMP	qPCR	qPCR	qPCR
Manufacturer		GeneReach	GeneReach	OptiGene	Tetracore	Indical	Genesig
Performance	Sensitivity	High	High	Moderate	High	High	High (LOD<100)
	Specificity	High	High	High	High	High	High
	Training needs	Moderate	Low	High	High	High	High
	Level of assessment	Evaluated by FAO	Peer-reviewed journal article	Peer-reviewed journal article	Peer-reviewed journal article	Peer-reviewed journal article	Evaluated by FAO
	References	Peer-reviewed publication not yet available	Tran *et al.* (2021)	Mee *et al.* (2020)	Liu *et al.* (2019)	Daigle *et al.* (2020); Elnagar *et al.* (2021)	Peer-reviewed publication not yet available
Advantages		Low-cost equipment	Full automatic, just load sample and go	No DNA extraction	Same as lab-based qPCR	Same as lab-based qPCR	Same as lab-based qPCR
		Battery-operated DNA automatic extraction	No training needed				
Disadvantages		Equipment: high cost					

**Validated in-house real-time PCR tests recommended by the OIE are King* et al. *(2003) and Fernandez-Pinero* et al. *(2013).*

***PCR Commercial Kits currently validated: INgene q PPA, INGENASA. 11.PPA.K.5TX/Q; Tetracore TC-9017-064; Virotype ASFV PCR Kit, INDICAL BIOSCIENCE; LSI VetMAXTM Thermo Fisher Scientific; IDEXX RealPCR ASFV Mix, IDEXX; ID Gene® African Swine Fever Duplex – IDVet; ADIAVET ASFV REAL TIME 100R, BIO-X DIAGNOSTICS. Commercial LAMP kit available from Geneworks (https://geneworks.com.au/; KIT-ASFV-96P).*

****Other sample types such as whole blood and tissues can be tested if DNA extraction is performed prior to LAMP testing (James* et al.*, 2010)*

VetMAXTM African Swine Fever Virus Detection Kit (Taqman® real time PCR) manufactured by Thermo Fisher Scientific LSI S.A.S. is included in the OIE Register of Diagnostic kits, www.oie.int/en/what-we-offer/veterinary-products/diagnostic-kits/the-register-of-diagnostic-kits/

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