ASF Diagnostics

Application of current diagnostic tests

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Australia's National Science Agency

Summary of ASF Diagnostics

- Range of virological and molecular methods are available to detect and characterise ASF virus
- **PCR is frontline test for outbreak investigations and routine diagnostics**
  - **Sensitive, specific, rapid**
- Antigen detection tests suffer from lack of Se, but are inexpensive and rapid
- Virus isolation relies on primary porcine cells, new sensitive cell lines needed

Laboratory diagnostic algorithm for ASF

Agent detection

Appropriate sample[1]

PCR (King, UPL etc)

Isolation (Primary cells – PBM, PAM)

Ag ELISA (In-house or INGENASA)

Pos

Pos

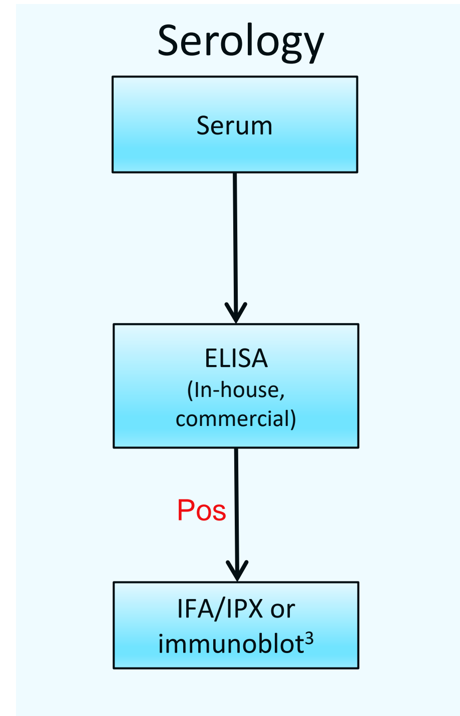
Pos

Conventional PCR[2] (genotyping)

Pos

Sequencing[2]

Gene targets: B646L (P72), B602L, I73R-I329L IGR etc



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

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 (Zsak)	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
Applied Biosystems	VP72	Realtime	N	VetMAX ASF kit
Indical	?	Realtime	N	*Virotype®* ASFV PCR

**Haines method ASFV/CSFV duplex*

Comparisons of Diagnostic performance - PCR

- Comparison of PCR tests using tissues from domestic pigs experimentally-infected with genotype I and II viruses (AAHL, unpublished)
- Methods reviewed
  - King et al 2003 (OIE)
  - Zsak et al 2005
  - Mckillen et al 2010
  - Haines et al 2013 (a multiplex with CSF)
  - UPL (INIA) (Fernandez-Pinero et al 2013)
  - VetMAX™ African Swine Fever Virus Detection Kit (Applied Biosystems)

Comparisons of Diagnostic performance - PCR

- Hands-on experience with clinical course, pathobiology, dynamics of shedding etc
- Allowed evaluation of AAHL's diagnostic capability
- Different sample types
  - Spleen; lymph nodes; liver; lung; sera; blood
  - Oral fluids
- At different sample time points during experimental infection
- Master mix volumes have also been assessed i.e. 25ul v. 15ul

Comparisons of Diagnostic performance

Comparison of PCR tests using tissues from domestic pigs experimentally-infected with genotype I and II viruses (AAHL, unpublished)

Tissue type	Genotype	Mean Ct*			
		King (OIE)	Zsak (USDA)	McKillen	Ingenasa
Lymph node	I	26.1	25.1	26.5	31.5
Spleen	II	20.0	18.7	20.1	24.9
Spleen	I	25.2	24.0	25.4	30.3
Lung	II	22.2	20.3	22.1	26.8
Liver	II	19.7	18.7	19.9	24.9
Uninfected spleen	NA	Undetected	Undetected	Undetected	Undetected
Spleen	II	19.8	19.3	20.5	25.3
Lung	I	28.9	27.5	29.4	35.1
Spleen	II	25.7	23.6	26.1	30.8
Spleen	I	29.1	28.2	29.6	35.1

King, Zsak and McKillen assays used AgPath-ID one-step RT-PCR reagents

sample type	Median	Min	Max	Range
spleen	27.2	26.7	29.6	2.9
	31.5	30.4	33.6	3.3
	35.2	33.5	37.3	3.9
EDTA blood	23.1	22.7	25.6	2.9
spleen	16.7	16.2	18.4	2.2
	30.0	29.1	32.3	3.3
	33.8	33.0	36.4	3.4
serum	19.2	16.7	21.1	4.3
EDTA blood	16.8	16.6	18.9	2.3
	21.0	19.7	22.9	3.3
	28.4	26.7	30.6	3.9
	29.1	28.2	31.1	3.0
	27.1	26.3	32.5	6.2
	30.6	30.0	36.2	6.1
IQC strong	23.5	22.3	26.0	3.7
IQC weak	31.7	30.9	33.9	3.0

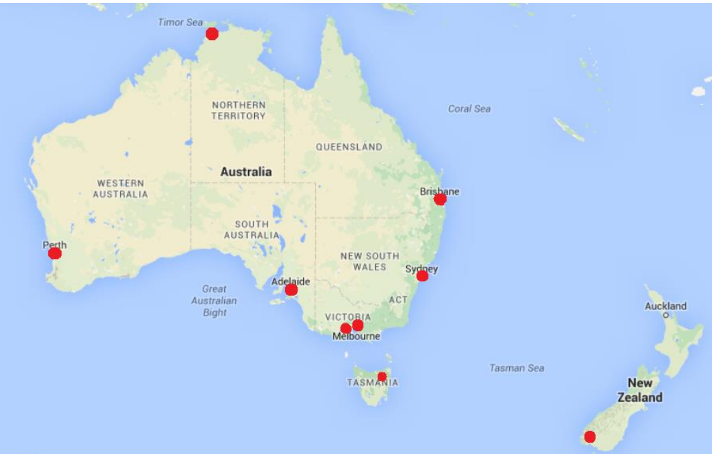
- King et al 2003
- Zsak et al 2005
- Mckillen et al 2010
- Haines et al 2013
- UPL (INIA)
- VetMAX™ ASFV

Variations seen relate to sample type and sample timeline.

General considerations

Assay	Pros	Cons
King et al 2003	• OIE recommended • Validation data at AAHL • FAO-preferred test, in use in SEA labs	Can be slightly less sensitive than others for low viral load samples
Zsak et al 2005	• Sensitive test • Validation data at AAHL	Not OIE 'recommended'
Haines et al 2013 (a multiplex with CSF)	• Sensitive test (slightly more than Zsak) • Referenced in OIE chapter (not 'recommended') • Multiplex with CSF	Limited validation data at AAHL
UPL (INIA) Fernandez-Pinero et al 2013	• Slightly lower CTs than Zsak • OIE recommended • Validated and recommended by the EU Ref lab	Limited validation data at AAHL Unusual probe
VetMAX™ ASFV Detection Kit (Applied Biosystems)	• OIE Biological Standards Commission reviewed – fit for purpose for virus detection in blood, serum tissues, est • Validation data available	Access to kit through procurement procedures may be limited

AAHL and LEADDR



Laboratories for Emergency Animal Disease Diagnosis and Response – LEADDR

- LEADDR has been running since 2009
- Participation Includes
  - Each state/territory represented by government veterinary laboratory
  - AAHL
  - New Zealand
- Through the provision of EQA AAHL support LEADDRs objective of **deliver of diagnostic capability** for identified significant diseases

Objectives of LEADDR

1. Establish a national system for the diagnosis of Emergency Animal Diseases (EADs) using standardized/harmonized laboratory testing services across a network of approved laboratories.

2. Establish a network-supported national surge capacity for EAD outbreak.

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Impacts of the program across the network

Implementation of National Proficiency Testin

PT assists with ongoing training which is

Participation in PT is

Essential to maintain diagnostic

Screening seized pork products for African swine fever virus

International movement of pork

- Implicated in long-range introductions
- Recent reports continue to highlight risk
- Seized at airports from travellers, imported illegally
- Targeted sampling of seized product for set periods for testing at AAHL for African Swine Fever Virus

African swine fever resilience

Product	Survival time
Meat with/without bone and ground meat	105 days
Salted meat	182 days
Cooked meat (min. 30 mins @ 70 °C)	0
Dried meat	300 days
Smoked and deboned meat	30 days
Frozen meat	1000 days
Chilled meat	110 days
Offal	105 days
Skin/Fat (also dried)	300 days
Blood stored at 4 °C	18 months
Faeces at room temperature	11 days
Putrefied blood	15 weeks
Contaminated pig pens	1 month

Review of import pathways

- examination of import conditions, to confirm they include measures to address the risk of ASF
- as a result, some import conditions have been modified:
  - increased processing for pigs ears and rawhide chews
  - suspended imports of personal consignments of pork jerky

Seized pork product testing

Three rounds of testing completed to date

- **ROUND 1:** 3rd to 17th of **December 2018** – to capture products being imported for Christmas
- **ROUND 2:** 21st of **January** to 3rd of **February 2019** – to capture products imported for Chinese New Year
- **ROUND 3:** 5th and 15th of **September 2019** (Awaiting final results)
- Products were collected from seized material from passengers at Melbourne and Sydney Airports as well as mail items from Sydney and Melbourne

Products seized were not eligible for importation into Australia

Types of Products

Testing undertaken

- A genetic test (PCR) was employed at AAHL to detect ASF virus DNA
  - clarified homogenate was extracted using the MagMAX-96 Viral Isolation Kit
  - Real-time PCR method Zsak et al 2005
- DNA sequence analysis was performed on a selection of samples that were positive in the PCR test.
- Virus isolation was attempted on select samples using primary porcine bone marrow cells (OIE, 2019)

PCR positives for ASF

ASF testing of seized pork products

450
400
350
300
250
200
150
100
50
0

60%
50%
40%
30%
20%
10%
0%

Round 1
Round 2
Round 3

Total number of complete ASFV tests
% of seized product positive

As a result

- In light of the changing distribution of ASF virus in Asia and parts of Europe, additional activities have been undertaken to ensure that biosecurity measures continue.
- Increased screening for banned pork products has been implemented at the border.
- Test results have shown that some of the pork products seized at our international airports and international mail centres were contaminated with ASF virus fragments.
- The test results reinforce the importance of continued risk management and compliance with Australia’s biosecurity requirements.

To date AAHLs role

- **Deliver of diagnostic capability** to the jurisdictional laboratories – ongoing over several years in SEA
- The provision of PT has resulted in **improved network harmonization of test methods and confidence in the network**.
- Backstopping missions – **assist, advise and troubleshoot** identified problems.
- **Development of EQA and PT training programs** for training missions in country – enabling laboratories to take a lead role
- **Distribution of emergency quality assured reagents** to south Asia and SE Asia country networks supported the objectives of FAO & OIE – ASF king et al 2003 PCR kits – 40K tests

Thank you

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Antigen detection tests - disadvantages

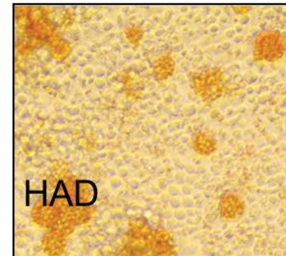
- Have low sensitivity for subacute and chronic cases of ASF due to the formation of Ab-Ag complexes in samples that interfere with assay
- *Therefore recommended as a 'herd test', and use together with other tests*

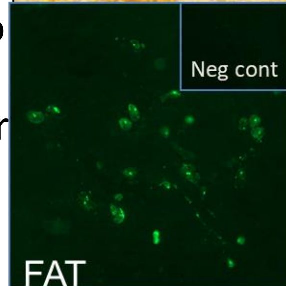
Table. Comparative sensitivity for analyzing positive field and experimental samples tested previously with UPL-PCR as a reference test (Gallardo et al. 2015).

Sample type	Ag-ELISA Ingenasa	
	No. of positive samples/total no.	Se (% [95% CI])
Experimental	76/92	82.6
Field	66/92	71.7
Total	**142/184**	**77.2 (70.6–82.6)**

Virus isolation

- Based on the inoculation of specimen onto primary porcine cells
  - Most sensitive substrate
  - Bone marrow (PBMC) or alveolar macrophages (PAM)
- Replication in 48-72 hours → CPE thereafter
- Virus detection:
  - Haemadorsption assay (HAD)
    - Very specific, but some strains are non-HAD
    - CPE+/HAD- : may be non-HAD ASFV or cytotoxicity of another virus → confirm by a second method
  - Immuno-detection: fluorescence antibody test (FAT) or peroxidase (IPX)
    - Highly specific and can identify non-HAD strains
  - Confirmation using PCR or Ag ELISA





Virus isolation

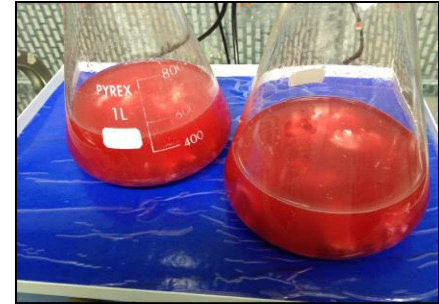
- Relatively sensitive compared to PCR for experimental samples and domestics pigs
- Lower sensitivity for wild boar samples (and cured pork)
  - Poor sample quality, degradation
- Example: comparison with PCR positive (UPL) samples (Gallardo et al. 2015)

Sample type	No. of positive samples*/total no.	% positive
Experimental	486/502	96.8
Field		
Domestic	29/34	86.0
Wild boar	27/91	30.7

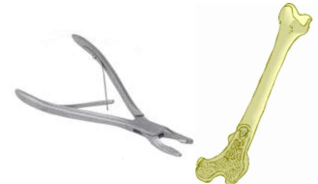
*After 3 passages

- Viable virus difficult to detect in high Ct samples (eg >35)

Primary porcine cells



- Gold standard for virus isolation (OIE)
- Disadvantages of primary cells:
    - ‘One shot’ use
    - Expensive and time consuming to produce
    - Variation in susceptibility to ASFV between batches/individual pigs
    - Ethics requirements
    - PAMs may contain co-infecting agents (eg SIV, mycoplasma)



- *Research goal: new sensitive* <u>*continuous*</u> *cell lines to replace primary cultures for virus isolation and for commercial development of live attenuated vaccines*

- The presence of representatives of the pig industry is to be noted. This is a good example of how Private-Public Partnerships (PPPs) can reinforce the effectiveness of veterinary service activities and support implementation of global programme for the control and the eradication of animal diseases

- https://www.oie.int/en/for-the-media/oie-public-private-partnerships/