Standard Laboratory Diagnostics and Networking in Asia

African Swine Fever

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Laboratory Networking
ASF Diagnostics
Regional ASF Networking

Laboratory Networks

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ECOHEALTH

Development of Veterinary Laboratory Networks for Avian Influenza and Other Emerging Infectious Disease Control: The Southeast Asian Experience

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- Emerging infectious disease (EID) control must be underpinned by effective laboratory services.
- Management of laboratories is best done through a network of support.
- Laboratory output can be ensured through quality assurance.

Active networks = enhance EID preparedness across the region.

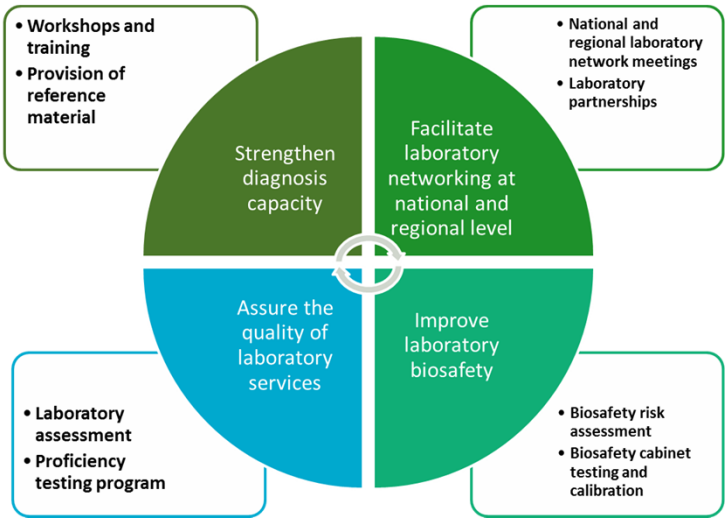
Why a Networked Approach?

- Networks leverage and link expertise, leading to efficiencies in use of resources & capabilities
- Networks optimize use of existing assets (equipment, buildings etc.) by connecting them to each other
- Networks can build remarkable capacities because they mobilize diverse and flexible individuals and organizations
- Networks offer a way to weave together and create capacities that get better leverage, knowledge, performance and results

Regional Network Activities

- **Targeted support for strengthening diagnostic capacity and laboratory management**
  - *Support PCR testing for multiple diseases*
  - *Standardization and validation of PCR protocols*
- **External Quality Assurance management (EQA) for diagnosis of targeted diseases**
  - *Support **proficiency testing** for priority diseases selected by the region*
- **Enhancing the role of Regional Leading/Support Laboratories for animal health**
  - *Build capacity in leading laboratories to design, implement a proficiency testing program for priority diseases*
- **Laboratory Twinning**

Technical Scope



The Aim of a PT Program is to:

- Identify variation between laboratories
- Maintain and improve analytical quality & inter-laboratory agreement
- Compare performance of different diagnostic assays

to provide **confidence** in results and processes, identify and **enable improvement**.

Objective of PT Program

- PT panel is designed to test the sensitivity and specificity achieved by each participating laboratory for molecular detection using PCR
- Access use of quality assurance – use of IQC
- Compare test harmonisation across the region
- Whole of assay approach – extraction, PCR method, interpretation and reporting
- Additional considerations for inclusion into PT panels is also based on;
  - the aims and objectives of the PT scheme
  - the participants competency and diagnostic expectations
  - the test being assessed (new vs. established)
  - whether the test is harmonised or standardised

The composition of the PT panel is designed to yield the maximum amount of information to enable the analysis of results and assessment of test performance

Benefits of PT participation

CONFIDENCE in Results

- Test methods are being followed
- Test results are accurate and precise
- Training is appropriate
- Systematic variations are identified
- Consistency between laboratories(harmonisation vs. standardisation)
- Credibility and compliance

Regional PT program 2007 to present

- The EQA program has involved a targeted approach to enable harmonized detection and response to emerging infectious diseases
  - new strains of avian influenza (serological and molecular)
  - Rabies (serological, antigen detection and molecular)
  - livestock production diseases of regional significance including CSF, PRRS and **ASF** (molecular)
- Building Regional Capacity of the National Laboratories for key Regional Diseases through external quality assurance.
  - Strengthen diagnostic capacity
  - Assure the quality of laboratory services

2018 Regional PT programme

- 25 participating laboratories across 19 countries

South East Asia

- Cambodia
- Indonesia (x4)
- Laos
- Malaysia
- Myanmar (x2)
- Philippines
- Thailand
- Vietnam (x2)

South Asia

- Bangladesh (x2)
- Bhutan
- India
- Nepal
- Pakistan
- Sri Lanka

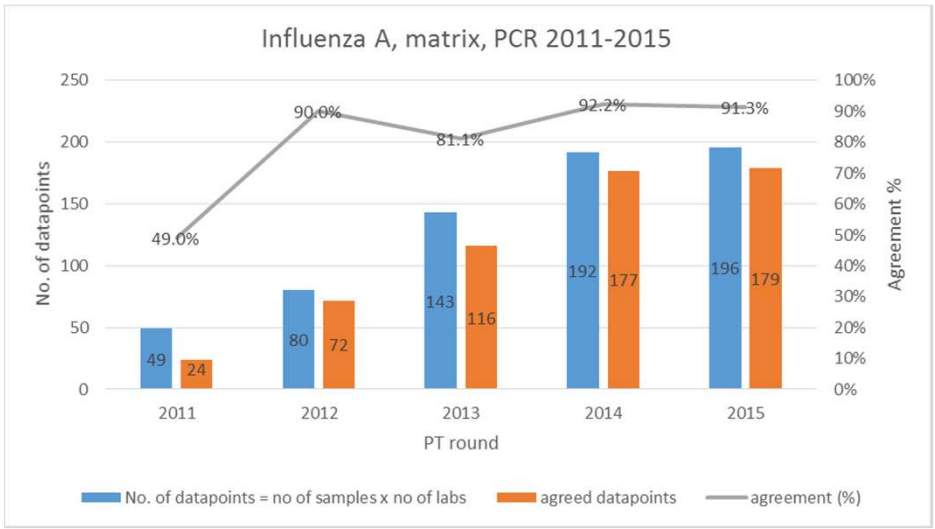
East Asia

- Japan
- Mongolia
- Taiwan (R.O.C)

Russia
New Caledonia

National laboratories designated within in each country to lead diagnosis of emerging/emergency infectious disease of significance.

- 21 laboratories reported results for proficiency testing
- Swine disease from 2011 (PCR CSF, ASF, PRRS and swine influenza)



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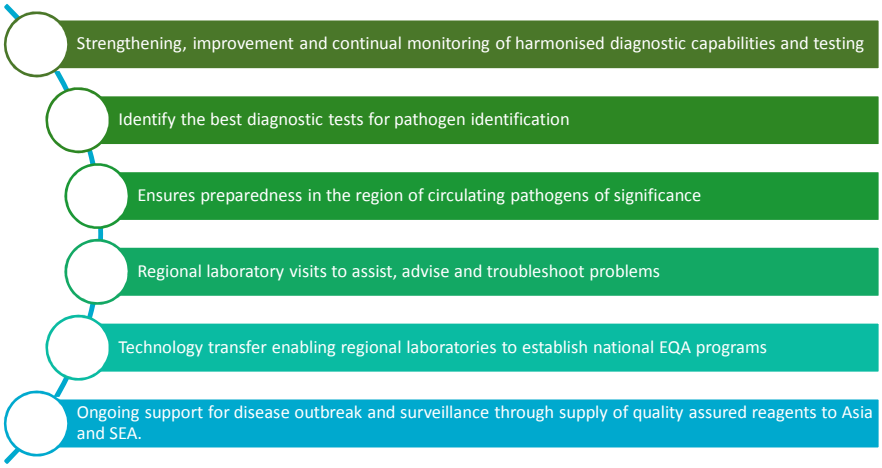
Value add activities - 'backstopping'

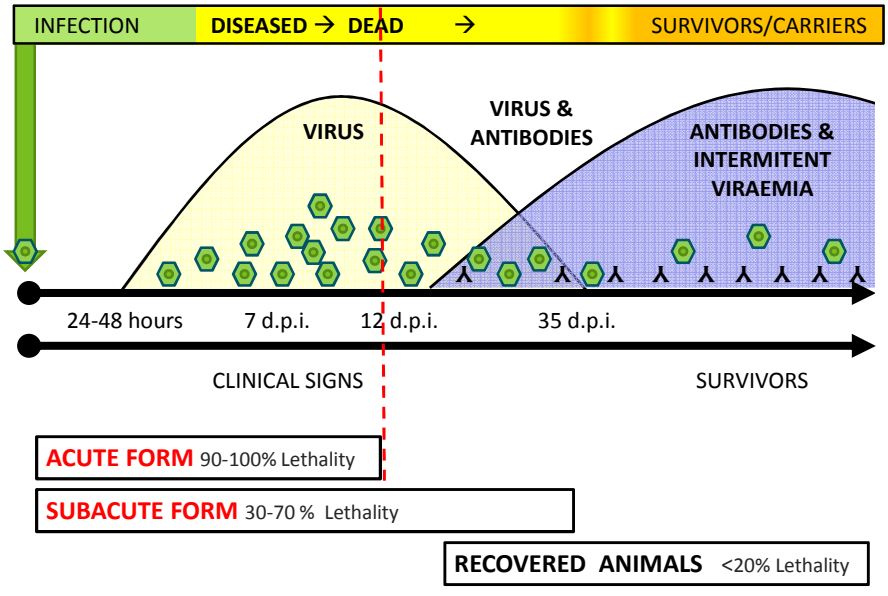
- A variety of activities have been established to augment the impact of the PT programs delivered into the SEA region.
- Scientists with expertise in a range of diagnostic techniques travel to participating laboratories to;
  - discuss PT results,
  - provide technical advice in a range of areas,
  - assess diagnostic laboratory spaces and practices, (e.g. biosafety, QA)
- The long-term goals are:
  - to assist laboratories and institutes in their transition to accreditation;
  - enable regional centres of excellence to conduct PT for their own satellite laboratories and for the region.

Laboratory services have been strengthened though an iterative process of monitoring, evaluating, reflecting and learning

Impacts of the network – QA program



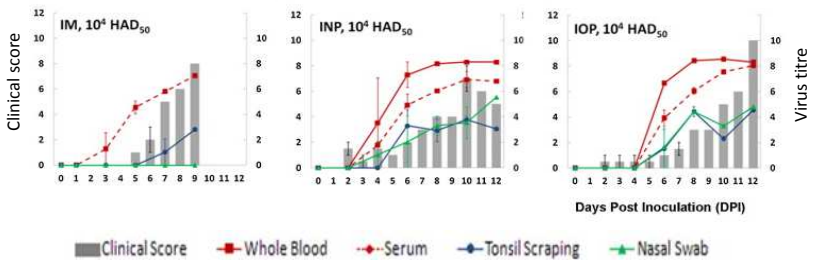
African Swine Fever Diagnostics



Source: CISA-INIA and UCM

Viremia and Shedding

- Highest levels of virus in blood
  - Viremia from 2-3 days after infection
- Levels in nasal and faecal swabs lower by 2-3 logs
- Virus in swabs usually detected earlier than in blood
- Virus shedding occurs ~2 days before clinical signs



Howey et al. (2013). Virus Res. 178(2):328

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Agent identification						
Virus isolation/ HAD test[1]	n/a	n/a	++	+++	++	n/a
FAT	n/a	n/a	++	++	+	n/a
ELISA for antigen detection	+	++	+	+	+	n/a
Conventional PCR	++	++	++	++	++	n/a
Real-time PCR	+++	+++	+++	+++	+++	n/a
Detection of immune response						
ELISA	+++	+++	+++	+	+++	n/a
IPT*	+++	+++	+++	+	+++	n/a
IFAT*	+++	+++	+++	+	+++	n/a
IBT*	++	++	++	+	++	n/a

Source: OIE

Diagnostic Specimens

- **Recommended** specimens:
  - Whole blood (serum), organs (especially spleen and lymph nodes, tonsils, kidneys)
- **Alternative** specimens:
  - Swabs: nasal, oral, faecal/faeces, fluid from the peritoneal cavity
  - Bone marrow (e.g. animals that have been dead for some time – best preserved tissue)

PCR

- Detection of partial gene fragments of the ASFV genome (*B646L* gene encoding p72)
- **Rapid** (5-6 hrs) and highly sensitive
- **Frontline choice for outbreak investigations** (peracute, acute infections) and **routine diagnostics**
- Can detect ASFV in absence of infectious particles or when at low levels
  - →Degraded or treated specimens (eg 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 (OIE)	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

**ASFV/CSFV duplex*

Comparisons of Diagnostic performance

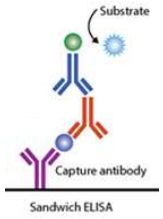
Comparison of PCR tests using tissues from domestic pigs experimentally-infected with genotype I and II viruses (AAHL, unpublished)

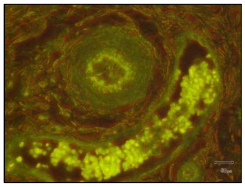
Tissue type	Genotype	Mean Ct*			
		King (OIE)	Zsak (USDA)	McKillen	Ingenasa
Lymph node	I	26.1	25.1	26.5	31.5
Spleen	II	20.0	18.7	20.1	24.9
Spleen	I	25.2	24.0	25.4	30.3
Lung	II	22.2	20.3	22.1	26.8
Liver	II	19.7	18.7	19.9	24.9
Uninfected spleen	NA	Undetected	Undetected	Undetected	Undetected
Spleen	II	19.8	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

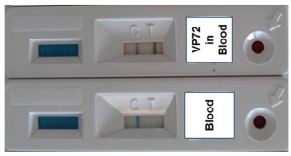
Antigen detection tests

- **Double sandwich ELISAs**
  - Inexpensive and useful for large scale testing
  - Do not require specialised equipment
  - Commercial ELISA available (Ingezim PPA DAS K2)
- **Direct FAT** of tissue impression smears or thin cryosections
  - Anti-ASFV Ab-FITC
- **Pen-side rapid test:** Ingenasa ASF CROM Ag one-step immuno-chromatographic test for blood samples
  - Detection of ASFV in blood samples
  - Comparable performance to ELISA
  - Se ~68%, Sp ~98% (vs UPL PCR)





http://asf-referencelab.info/asf



Sastre et al. 2016 BMC Vet Res.

Antigen detection tests - disadvantages

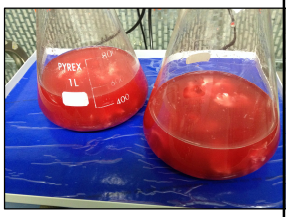
- Have low sensitivity for subacute and chronic cases of ASF due to the formation of Ab-Ag complexes in samples that interfere with assay
- *Therefore recommended as a 'herd test', and use together with other tests*

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 - 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 continuous cell lines to replace primary cultures for virus isolation and for commercial development of live attenuated vaccines*

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

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

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
- Serology useful for surveillance (including proof of freedom)

ASF Regional Networking

Swine Diseases Regional PT

- Swine diseases have been a focal point in regional program (FAO supported PT and backstopping) since 2011
- Panels include ASF, CSF, PRRS, Swine Influenza (and negative) samples
- In 2019 – 28 laboratories from 20 countries enrolled
  - 24 labs supported by FAO, 3 by OIE)
  - 25 laboratories submitted results

ASF Regional Support

- In late 2018 ASF PCR kits (primers, probes, strong and weak extraction controls) provided for regional preparedness
- 10 kits to FAO – distributed to SEA countries
- 10 ASF kits to NIAH, Bangkok (through OIE Twinning Project)
- 10 ASF kits to RAHO6, HCMC Vietnam (through OIE Twinning Project)
- Standing Group of Experts on ASF in Asia (OIE, FAO)
  - Collaboration, exchange of information

NIAH/AAHL OIE Laboratory Twinning

Priority Area	Situation at Commencement	Activity Summary	Situation at Completion
Virus Isolation and conventional testing techniques for target diseases	Good existing technical expertise. Need for expanded range of cell lines especially for ASF	Cell lines provided and specific test (VNT and IFX) training provided through NIAH staff visits to AAHL	Expanded cell lines and established confirmatory immuno-tests including for ASF and PED.
PCR Protocols and primers/probes reviewed and updated where required	Good comprehensive range of PCR capabilities.	NIAH participates annually in FAO supported and AAHL managed Regional PT rounds for avian and for swine diseases, which objectively measure their performance. NIAH uses the PT results to alert them to when corrective actions under their Quality system may be required.	Ongoing high levels of test performance achieved under the Regional PT rounds
Review Serology Tests	A comprehensive range of commercial ELISA tests existed. The PED serology tests were reviewed and comparative testing conducted with AAHL	NIAH is now developing new tests or improving some ELISA tests as part of applied research projects for their staff. Training on test validation techniques for new or improved tests was conducted. Training of IFA for ASF was conducted	NIAH has the expertise to develop or improve a range of serology tests (ELISA, CFT, IFA and MAT for Lepto) and to undertake test comparisons and test validation analyses to determine test performance parameters including diagnostic sensitivity/specificity and repeatability

Thank you

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AAHL Virology team

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Food and Agriculture Organization of the United Nations

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