



USAID
FROM THE AMERICAN PEOPLE



**Report from the Regional Laboratory
Network Training Workshop and
Activities on the Diagnosis of Influenza
H7N9 and Pig Diseases: Africa Swine
Fever (ASF), Classical Swine Fever (CSF)
and Porcine Reproductive and
Respiratory Syndrome (PRRS).**

**29 July - 9 August 2013
Regional Animal Health Office – 6,
Ho Chi Minh City, Viet Nam.**

**Chris Morrissy, Julie Cooke
and Frank Wong**

Table of Content

	List of Acronyms	4
1	Executive Summary	5
2	Background	6
3	Objectives	7
4	Participants	8
5	Expected Outputs	8
6	Main Findings	8
6.1	H7N9 Influenza A and Pig Diseases: ASF, CSF and PRRS Training Workshop, Ho Chi Minh City, Regional Animal Health Office – 6, Viet Nam.	9
	• Objectives	10
	• Workshop Activities:	10
	• Regional protocols for diagnosis and characterization of H7N9, ASF, CSF & PRRS.	11
	– <i>Influenza (including H7N9) Diagnostic Test Algorithm</i>	11
	– <i>ASF Diagnostic Test Algorithm</i>	12
	– <i>CSF Diagnostic Test Algorithm</i>	12
	– <i>PRRS Diagnostic Test Algorithm</i>	13
7	Training and workshop evaluation	14
8	Conclusion and Recommendation	15
Annex 1	Agenda of the H7N9 Influenza A, ASF, CSF and PRRS training workshop, 29 July - 9 August 2013	17
Annex 2	Participants list of the ASF, CSF and PRRS Training Workshop	24
Annex 3	Weekly Agenda of the Rabies & NDV Training Workshop, 6 – 17 August 2012	27
Annex 4	SEA Regional Laboratory Network: Laboratory Diagnostic Test Algorithms for Avian Influenza A Surveillance and Investigation	30
Annex 5	ASF Diagnostic Test Algorithm for Surveillance and Investigation	33
Annex 6	PRRS Diagnostic Test Algorithm for Surveillance and Investigation	35

Annex 7	CSF Diagnostic Test Algorithm for Surveillance and Investigation	37
Annex 8	Pre/Post Training Workshop Laboratory Questionnaire	39
Annex 9	Workshop Evaluation Form	44

Attachments:

1. Regional SOPs (see Regional SOP file)
2. Workshop documents and presentations (see Workshop Report file)
3. Course Evaluation from the Regional Training on Diagnosis of H7N9, ASF, CSF, and PRRS
4. Minimum Requirement for Diagnostic Laboratory Biosafety and Quality Assurance for Veterinary Laboratory Processing Animal Influenza Samples
5. Minimum Requirement for a Diagnostic Laboratory Biosafety at each level in the Regional Laboratory Network

List of Acronyms

AAHL	Australian Animal Health Laboratory
AI	Avian Influenza
ASEAN	The Association of Southeast Asian Nations
ASF	African Swine Fever
BSCII	Biological Safety Class 2 Cabinet
BSL	Biosecurity Level
CSF	Classical Swine Fever
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CT	Cycle Number Threshold
DAH	Department of Animal Health
DLD	Department of Livestock Development
EPT	Emerging Pandemic Threat Program
FAO	Food and Agriculture Organization of the United Nations
FAT	Fluorescent Antibody Test
HI	Haemagglutination Inhibition test
HPAI	Highly Pathogenic Avian Influenza
HPEDs	Highly Pathogenic Emerging Diseases
IPX	Immuno-peroxidase test
IQC	Internal Quality Control
LIMS	Laboratory Information Management System
ND	Newcastle Disease
NDV	Newcastle Disease Virus
NIAH	National Institute Animal Health
NPLA	Neutralising Peroxidase Linked assay
OIE	World Organization for Animal Health
PCR	Polymerase Chain Reaction
pH1N1	Pandemic Influenza A H1N1 2009
PRRS	Porcine Reproductive and Respiratory Syndrome
PPE	Personal Protective Equipment
PM	Post-mortem Examination
PT	Proficiency Testing
QA	Quality Assurance
QC	Quality Control
Rabies RFFIT	Rabies Rapid Fluorescent Foci Inhibition Test
Rabies FAVN	Rabies Fluorescent Antibody Virus Neutralization test
Rabies PAVN	Rabies Peroxidase Antibody Virus Neutralization test
RAHO – 6	Regional Animal Health Office – 6 Ho Chi Minh City
RRL	Regional Reference Laboratory
RT-PCR	Reverse Transcription Polymerase Chain Reaction
rRT-PCR	Real-time RT-PCR
SARS	Severe Acute Respiratory Syndrome
SOP	Standard Operating Procedures
TADs	Transboundary Animal Diseases
USDA	United States Department of Agriculture
USAID	United States Agency for International Development
VI	Virus Isolation
WHO	World Health Organization

1. Executive Summary

The Australian Animal Health Laboratory and FAO collaborated with Department of Animal Health (DAH) Viet Nam to organize regional laboratory training workshops at the Regional Animal Health Office No. 6 (RAHO – 6) Ho Chi Minh City, Viet Nam. The laboratory-based training workshop was organized under the FAO Regional Laboratory Networking Strategy and were specifically designed to strengthen regional laboratory diagnostic and investigation capacities. The training workshop focused on priority animal diseases; Influenza (primarily H7N9 and pig diseases, Africa Swine Fever (ASF), Classical Swine Fever (CSF) and Porcine Reproductive and Respiratory Syndrome (PRRS).

The training activities had an emphasis on hands on training in the diagnosis and characterisation of these priority and emerging diseases according to the agreed regional diagnostic algorithms with a focus on interpretation and troubleshooting of test results. The workshop gave the opportunity to carry out training for Influenza A H7N9 from China to support this regional emergency after outbreaks in China which lead to human deaths from LPAI H7N9 Influenza A virus. The workshop followed the Regional Influenza diagnostic algorithmic with training in serology, identification by Virus Isolation (VI) and Haemagglutination Assay (HA) and Haemagglutination Inhibition test (HI) and Real-time PCR for Influenza A H7N9 from China. The workshop also reviewed diagnosis for pig diseases with a focus on problems identified in the 2012 IDENTIFY PT round. The workshop encompassed techniques and procedures that can be applied generically to the characterization of a wide range of agents.

The training in HCMC covered updated the diagnosis of key regional pig diseases of CSF and PRRS with a focus on the diagnosis of ASF which has become a threat for the region. ASF and H7N9 were used to demonstrate how laboratories should implement new tests into their laboratory. In the distribution of the reagents for H7N9 a number of laboratories had problems in setting up the PCT tests for H7 & N9 in their laboratories and these problems were reviewed as part of troubleshooting exercises in the workshop.

The training covered the options for diagnosis of pig diseases and the importance of combining laboratory diagnosis with activities in the field, rapid reporting, data collection, quarantine and movement control for disease control. The participants were given hands on experience in all tests used for diagnosis of pig diseases, PCR, VI and cell culture, Fluorescent Antibody Test (FAT)/ Immunoperoxidase (IPX) test, antigen ELISA and serology using FAT/IPX, virus neutralisation test (VNT)/ NPLA and ELISA.

The training highlighted the problems of introducing new tests into a laboratory, especially in the emergency situation with H7N9, how important Internal Quality Control (IQC), the use of reference positive and negative controls, are in setting up a new test in the laboratory and verifying that test is working correcting and giving maximum sensitivity and specificity needed for surveillance (especially for LPAI where birds are healthy and virus titres are low). The workshop covered the use of the HA & HI tests for identification and serology showing how the H7N9 antigen and

antiserum were not specific to H7N9 and only indicated the presence of a H7 Influenza and not that it was H7N9.

The training covered all the required diagnostic tests including QA and Biosafety & Biosecurity requirements. Procedures for test optimization/verification that a new test is working in the laboratory along with troubleshooting were discussed at length as with the updated the SOPs for PCR to ensure they were written so the countries were able to do the tests from the methods (i.e. written with enough detail and with wording that was easy to understand).

The training activities again gave networking opportunities for the participants to strengthen regional laboratory links with each other and RAHO6 as a leading regional laboratory.

The Laboratory Training Workshop was well received by all participants and formal feedback confirmed that the laboratory activities were highly successful. All participants were given PT panels and reagents to take back with them to their laboratory. The outcomes from the PT panels will be followed up in the back-stopping missions (see PT and country reports for outcomes). The back-stopping missions were seen as an important follow-up to the workshops and separately as a chance for the laboratory to ask questions about all diagnostic tests at the laboratory and to establish the tests in their environment. Back-stopping missions helped with training all staff and were a chance for laboratories to ask questions and solve problems for all laboratory activities not just the priority diseases.

RAHO-6 staff in particular were mentored in the requirements for running a workshop which included preparation for the workshop and modifications to the program to deal with unexpected problems.

The workshops allowed all participants to gain hands on experience and allow them to gain trouble-shooting experience especially in setting up a PCR test. Virus isolation, serology and Identification was another area where hands on experience were important to the participants. Interpretation of test results and the use of positive and negative controls (IQC) was another area covered by the workshop that was valuable to participants; participants often just report results without understanding them. Interpretation and reporting laboratory test results along with the field information, with a recommendation to the field veterinarians is an area where all laboratories need assistance and training.

The information from the workshop was supplied on the participant USB.

2. Background

Organisms circulating in the domestic and wild animal populations can potentially pose a threat to both animal and human health as the relationship between animal infectious diseases and emerging human diseases is now well established. The changes in ecosystems resulting from human activity may result in the emergence and spread of novel pathogens coupled with the increased risk of exposure to previously unknown pathogens resulting from increased human activities in areas that are only sparsely populated. The impact of these Highly Pathogenic Emerging

Diseases (HPEDs) on health and livelihoods, either in humans or livestock, cannot be exaggerated as seen from Severe Acute Respiratory Syndrome (SARS), Highly Pathogenic Avian Influenza (HPAI) and pandemic H1N1 influenza. Because the majority of emerging diseases in humans originate from animals, both the animal health and human health sectors have an interest in, and responsibility for, monitoring and controlling these pathogens.

Many parts in Asia have shown to be hotspots of HPEDs due to a variety of contributing factors. The region has the highest rate of human and animal population growth in the world. The farming systems are rapidly intensifying with often poor biosecurity. Forests are being rapidly encroached and large populations of domestic livestock and dense human populations are increasingly coming into close contact with wild animals and their alien pathogens. Thus, it is expected that HPEDs with epidemic and pandemic potential in animals and humans will regularly emerge in the region, threatening the global community. While HPEDs may emerge in any one of the countries in the region, it is imperative that HPEDs are addressed on a regional basis given their transboundary nature.

Using a One-Health Approach, the World Organization for Animal Health (OIE), the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) are coordinating global activities to address health risk at human-animal-ecosystem interface through multi-sectoral cooperation and strong partnership. Currently, two programs allow the tripartite organization (FAO, OIE, WHO) to address emerging infectious diseases in Asia including the Emerging Pandemic Threat Program (EPT) supported by the United States Agency for International Development (USAID) and the Regional Collaborative Program on HPEDs supported by the European Commission. Despite different approaches to addressing emerging infectious diseases, both programs recognize the significance of the Regional Laboratory Network in supporting surveillance and response to disease outbreak.

Key implementation strategy of regional laboratory network activities was to use a programmatic approach by pooling resources from various projects to support every activity under the regional laboratory networking strategy, which allow ECTAD-RAP to cover all member countries in the region while minimizing effort and maximize outputs and outcomes. To facilitate the programmatic approach to regional laboratory networking, a SE Asia regional laboratory networking strategy was developed based on FAO-OIE-WHO global laboratory networking strategy including; 1) Assessment of existing laboratory capacity in the region, 2) Expanding laboratory networks across human and animal health laboratories, 3) Strengthening laboratories diagnostic and investigation capacities and 4) Expanding platform for information and biologic materials sharing.

3. Objectives

- Strengthen animal health laboratories capacity to diagnose: Influenza, Influenza H7N9, ASF, CSF & PRRS
- Sharing update information on disease situation in the region and update diagnostic techniques

- Discussion on regional Animal Influenza, Rabies, ND, ASF, CSF and PRRS control, early detection of emerging infectious diseases in animal and capacity building for laboratory diagnosis of priority and emerging diseases.

4. Participants

Participants from each of the national veterinary laboratories in Cambodia, Indonesia, Lao PDR, Philippines, Malaysia, Myanmar, Thailand and Viet Nam took part in the training (See Annex 2).

5. Expected Outcomes

- Training
 - Animal health laboratories are capable of consistently and accurately diagnose Influenza, H7N9, ASF, CSF and PRRS.
- Participants trained in Serological Tests for the detection of antibodies in serum.
- Participants trained in the detection of virus/antigen/genome in field samples and from virus isolates using different detection systems according to laboratory capability.
- Ability to conduct a RT-PCR
- Trouble shooting strategies
- Ability to choose an appropriate testing program for a particular purpose (Fitness of the test for purpose)
- Delivery of proficiency panels to each laboratory according to the capability established with the aid of the Laboratory capability questionnaire these results are due 27th September 2013
- Participants with a general overview of the requirements of a QA system (ISO17025) and Biosafety and Biosecurity for the laboratory and refresher training building on previous activities.

6. Main Findings

The workshop followed the Regional diagnostic algorithms for Influenza, ASF, CSF & PRRS with training in serology, identification by Virus Isolation (VI) and Real-time PCR for pig diseases (focus on ASF) and Influenza A H7N9 from China. The workshop also reviewed diagnosis for pig diseases with a focus on problems identified in the 2012 IDENTIFY PT round and for influenza the problems associated with the setting up of H7N9 tests. The workshop encompassed techniques and procedures that can be applied generically to the characterization of a wide range of agents.

The information from the training workshops on Rabies and ND and H7N9, ASF, CSF & PRRS from the countries highlighted the continued need for harmonisation of diagnostic tests in the region and the need for ongoing PT to ensure that the results from tests are correct and consistent. The countries use different PCR and serology protocols, reagents and QA controls which make it difficult to compare the results obtained in each laboratory. There is an ongoing need for regional PT and a need for regional QA reference controls (regional reference controls: low positive control) for

each test run to allow comparison among test results from each laboratory. This allows the different tests to be compared as the result to the Regional Reference control can be compared and allow laboratories to change tests to another test used in the region or to the regional test SOP if their test is found to be not performing.

The budget for reagents remains a big problem for some countries to put new tests in place and for the field work to collect samples to use tests. The training allowed hands on practice and gave participants the guidelines for H7N9, ASF, PRRS and CSF diagnosis, the workshop discussions provided the current situation in the region for H7N9, ASF, CSF and PRRS and future directions and gaps. The workshop presented a diagnostic test algorithm for each disease for disease investigation and surveillance (see Annex 4, 5, 6 & 7) and a recommended test SOP (provided as attachments to this report). Regional SOPs were based on AAHL SOPs which are validated and based on OIE recommended methods.

The workshops highlighted the importance of QA and the need for the establishment of a QA system in each country laboratory network and the ongoing need for Biosafety and Biosecurity training to establish a culture of safety. Some laboratories, e.g. RAHO – 6 & NIAH Thailand, have accreditation to ISO17025 and others have no quality system in place Laos PDR and Cambodia. A QA system gives a laboratory a structure to implement test QA, and ensure the laboratory has the systems (written in SOPs) in place to ensure tests are giving the correct answers and staff are working safely.

Occupational Health and Safety (O.H& S) especially the use of PPE when working with hazardous chemicals is not practiced in almost all regional laboratories. The countries varied in their commitment to establishing a laboratory QA system and a safety culture for Biosafety and O.H & S.

6.1 H7N9, ASF, CSF and PRRS Training Workshop, Ho Chi Minh City, Regional Animal Health Office – 6, Viet Nam.

The training in HCMC covered the key regional pig diseases, ASF, CSF and PRRS focusing on the diagnostic tests required for diagnosis of ASF which has become a threat for the region. The workshop gave the opportunity to carry out training for Influenza A H7N9 from China to support this regional emergency after outbreaks in China which lead to human deaths from LPAI H7N9 Influenza A virus.

The training highlighted the problems of introducing new tests into a laboratory, especially in the emergency situation with H7N9, how important Internal Quality Control (IQC), the use of reference positive and negative controls, are in setting up a new test in the laboratory and verifying that test is working correctly and giving maximum sensitivity and specificity needed for surveillance (especially for LPAI where birds are healthy and virus titres are low). The workshop covered the use of the HA & HI tests for identification and serology showing how the H7N9 antigen and antiserum were not specific to H7N9 and only indicated the presence of a H& Influenza and not that it was H7N9.

The training covered the options for diagnosis of pig diseases and the importance of combining laboratory diagnosis with activities in the field, rapid reporting, data collection, quarantine and movement control for disease control. The participants were given hands on experience in all tests used for diagnosis of pig diseases, PCR, virus isolation (VI) and cell culture, FAT/IPX, antigen ELISA and serology using FAT/IPX, virus neutralisation test (VNT)/ NPLA and ELISA.

The training at RAHO – 6 covered pathology, cell culture, virology and serology for disease diagnosis, as well as Quality Assurance (QA), including test optimisation and Biosafety & Biosecurity. The training covered all the required diagnostic tests including QA and Biosafety requirements.

Objectives

To review the diagnostic concept of the available diagnostic assays, and provide hands-on training on diagnosis of ASF, CSF and PRRS under an ISO17025 framework.

Workshop Activities:

- i. ASF, CSF and PRRS overview
 - Current disease situation for ASF, CSF & PRRS
 - ASF, CSF and PRRS Diagnosis
 - ASF, CSF and PRRS Diagnosis Algorithm
- ii. Practice on Virus Isolation in eggs for H7N9
 - Passages needed/SOP
 - Identification of H7N9 by HA/HI
 - Also used H5 & NDV
 - Use of Reference serum and antigens (QA & IQC)
 - Eggs to use?
 - Storage of isolates
- iii. Practice on serological identification of H7N9
 - Use AAHL Type A ELISA as a screen test
 - Set-up HI test serology
 - Cross titrations using different H7 Reference antigens and serum
 - Also used H5 & NDV
- iv. Practice on Optimisation of H7N9 & ASF Real-time PCR
- v. General overview of the requirements of ISO17025
 - Use of positive and negative controls
 - Test Coversheets, reagent monitoring and recording
- vi. General overview of the requirements for Biosafety and Biosecurity
- vii. Trouble shooting strategies were covered for the different tests
- viii. Delivery of Proficiency panels

Regional protocols for diagnosis and characterization of H7N9, ASF, CSF & PRRS.

Influenza (including H7N9) Diagnostic Test Algorithm:

The Diagnostic test algorithm for Influenza (Annex 4) outlines the diagnostic process for influenza surveillance and investigation including H7N9. The recommended samples for an Influenza investigation are swabs and tissues; the samples should be taken individually and not pooled. Avoid pooling samples in the field whenever possible; where it is required for testing purposes, it is best done at the laboratory by combining a maximum of 5 similar samples per pool from the same sample type, species, and epidemiologic unit.

For influenza the testing starts with a Influenza Type A Real-time PCR to detect all viruses and then follows with a specific Real-time RT-PCR for H Type (e.g. H7 & H5) and N Type (N9 & N1). The recommended tests for detection of Influenza H7 and N9 antigen/genome using primers/probes that have been validated and optimized to give maximum sensitivity and specificity. The recommended regional protocol for Influenza TaqMan Assays are attached for reference, it is based on the AAHL SOP.

All positive individual samples or a range of samples, as required, should be send sent to a Reference or key laboratory for VI and further characterization. All laboratories should have the capability to carry out Realtime RT-PCR and the capability to produce cDNA. Production of cDNA allows laboratories to send cDNA, where samples cannot be sent, for sequence analysis at OIE regional reference laboratory. Key regional and reference laboratories need to have the capability for VI and molecular characterization. Whole genome sequencing will be carried out at the reference laboratory to establish regional sequence database of circulating virus. Whole genome sequencing may be important in identifying changes to the Influenza virus isolates and where the virus came from.

Virus Isolation is also used for detection of Influenza virus, VI is as sensitive or more sensitive to Real-time PCR but takes longer (up to 2 weeks) and requires having the correct references reagents for HA/HI. Also working with VI means a higher risk for exposure to virus when doing VI. The choice of tests depends on the purpose the test is to be used for, VI is often necessary for further characterisation.

Serology for Influenza uses an ELISA and or HI test to detect Influenza antibody. The AAHL Type A ELISA was used in this workshop but other ELISA are available e.g. IDEXX ELISA. The Type A Influenza ELISA tells you if the animal has influenza antibody from any influenza virus/H Type. The HI test is used to tell if you have antibody to a specific H Type e.g.H7 antigen is used to confirm present of H7 antibody but using a H7N9 antigen and getting a positive does not necessary mean your antibody is to H7N9.

ASF Diagnostic Test Algorithm:

The Diagnostic test algorithm for ASF (Annex 6) outlines the diagnostic process for ASF surveillance and investigation. The recommended samples for an ASF investigation are spleen, blood, serum, lymphoid tissue, lung and nasal swab; the samples should be taken individually and not pooled. Avoid pooling samples in the field whenever possible; where it is required for testing purposes, it is best done at the laboratory by combining a maximum of 5 similar samples per pool from the same sample type, species, and epidemiologic unit.

A specific Realtime RT-PCR is the recommended test for detection of ASF antigen/genome using primers/probes that have been validated and optimized to give maximum sensitivity and specificity.

The recommended regional protocol for ASF TaqMan Assay is attached for reference, it is based on the AAHL SOP. All positive individual samples or a range of samples, as required, should be sent to a Reference or key laboratory for VI and further characterization. All laboratories should have the capability to carry out Realtime RT-PCR and the capability to produce cDNA. Production of cDNA allows laboratories to send cDNA, where samples cannot be sent for sequence analysis at an OIE regional reference laboratory. Key regional and reference laboratories should have the capability for molecular characterization.

Serology for ASF uses an ELISA to detect ASF antibody, serology using IPX or FAT is generally only available in Reference laboratories as it requires cell culture technology.

The training workshop provided training in immunoperoxidase staining for CSF for identification of CSF and also in the NPLA for detection of CSF antibodies. The use Immunofluorescence Test (FAT) was demonstrated during the training workshop. The techniques used for CSF are the similar to those used for ASF. CSF was used to demonstrate the techniques for CSF, ASF & PRRS.

CSF Diagnostic Test Algorithm:

The Diagnostic test algorithm for CSF (Annex 7) outlines the diagnostic process for CSF surveillance and investigation. The recommended samples for a CSF investigation are spleen, blood, serum, lymphoid tissue, lung and nasal swab; the samples should be taken individually and not pooled. Avoid pooling samples in the field whenever possible; where it is required for testing purposes, it is best done at the laboratory by combining a maximum of 5 similar samples per pool from the same sample type, species, and epidemiologic unit.

A specific Real-time RT-PCR is the recommended test for detection of CSF antigen/genome using primers/probes that have been validated and optimized to give maximum sensitivity and specificity.

The recommended regional protocol for CSF TaqMan Assay is attached for reference, it is based on the AAHL SOP. All positive individual samples or a range of samples, as required, should be sent to a Reference or key laboratory for VI

and further characterization. All laboratories should have the capability to carry out Realtime RT-PCR and the capability to produce cDNA. Production of cDNA allows laboratories to send cDNA, where samples cannot be sent, for sequence analysis at OIE regional reference laboratory. Key regional and reference laboratories need to have the capability for VI and molecular characterization. CSF VI and identification protocols were made available to the participants of the workshop. Whole genome sequencing will be carried out at the reference laboratory to establish regional sequence database of circulating virus. Whole genome sequencing may be important in identifying changes to CSF virus isolates and where the virus came from.

Antigen detection ELISAs are also used for detection of CSF antigen/virus, these ELISAs are less sensitive than Real-time PCR but are useful for detect of CSF. The antigen detection ELISA is best used for disease investigation where there are sick animals and higher amounts of virus, while the PCR is best used for surveillance where virus levels are lower e.g. detection of carrier animals. The choice of tests depends on the purpose the test is to be used for.

Serology for CSF uses an ELISA or Virus Neutralisation Test (VNT) which detects CSF antibody, the Prionics or IDEXX ELISA is the ELISA kit most used. Other CSF ELISA kits are available for use and all kits need to be validated before use. The VNT or NPLA is generally only available in Reference laboratories as it requires cell culture technology.

The training workshop provided training in immunoperoxidase staining for CSF for identification of CSF and also in the NPLA for detection of CSF antibodies. The CSF NPLA is used as a confirmation test and is also important for determining post-vaccination titres and maternal antibody titres. The Immunofluorescence Test (FAT) was demonstrated during the training workshop.

PRRS Diagnostic Test Algorithm:

The Diagnostic test algorithm for PRRS (Annex 5) outlines the diagnostic process for PRRS surveillance and investigation. The recommended samples for a PRRS investigation are blood, serum, spleen, lung, lymphoid tissue and nasal swab; the samples should be taken individually and not pooled. Avoid pooling samples in the field whenever possible; where it is required for testing purposes, it is best done at the laboratory by combining a maximum of 5 similar samples per pool from the same sample type, species, and epidemiologic unit.

A specific Real-time RT-PCR for US & EU PRRS is the recommended test for detection of PRRS antigen/genome using primers/probes that have been validated and optimized to give maximum sensitivity and specificity. Primers and Probes specific to Chinese PRRS can be used for further characterization of the US PRRS positive samples, confirming the presence of the new Chinese PRRS virus.

The recommended regional protocol for PRRSV TaqMan Assay is attached for reference, it is based on the AAHL SOP. All positive individual samples or a range of samples, as required, should be sent to a Reference or key laboratory for VI and further characterization. All laboratories should have the capability to carry out rRT-

PCR and the capability to produce cDNA. Production of cDNA allows laboratories to send samples for sequence analysis at OIE regional reference laboratory. Key regional and reference laboratories need to have the capability for VI and molecular characterization. PRRS VI and identification protocols were made available to the participants of the workshop. Whole genome sequencing will be carried out at the reference laboratory to establish regional sequence database of circulating virus. Whole genome sequencing is more important in identifying changes in PRRS.

Serology for PRRS uses an ELISA which detects PRRS antibody, the IDEXX ELISA is the ELISA kit most used. Other PRRS ELISA kits are available for use and all kits need to be validated before use. For PRRS serology is often used to identify a farm that has PRRS.

The training workshop provided training in immunoperoxidase staining for PRRS for identification of PRRS and also in the PRRS immunoperoxidase assay (PRRS IMPA) for detection of PRRS antibodies. The PRRS IMPA is used as a confirmation test and also in further characterization of PRRS antibody.

7. Training and workshop evaluation

A set of test questions and an evaluation form (Annex 8 & 9) was given to each participant after the training and workshop. The results from the evaluation for the training workshops are attached to report.

All the participants agreed that the training workshop was useful and were satisfied with the workshop and training. The general feedback included: the hands on training in this workshop was very useful, the discussion sessions for problem solving were very useful and RAHO-6 staff were excellent and working with other countries was useful for us to compare problems.

The problems which may limit their application of the techniques trained include; government or administrative issues, lack staff or lack of opportunity to use training, lack of budget or funding and lack of reagents or domestic supplier.

Participants provided many questions showing their interest in the workshops activities. Their familiarity with the testing protocols (HA/HI) and its specificity increased during the workshop, allowing these participants to take back to their countries added knowledge about the HI technique for indentifying H7N9.

Some participants were from different laboratory sections, and so, had no prior experience with HI's. This provided an opportunity to go back to basics for everyone, reinforcing the importance of IQC and enhancing everyone's techniques in the process.

The mentoring with these countries will continue through email conversations There were delays due to lack of equipment but having a facility that allowed all participants to have enough equipment is difficult e.g. microscopes for examining cells.

8. Conclusions and recommendation

The workshop participants agreed there was a harmonised approach to disease diagnosis and the implementation of QA in the laboratories. The regional guidelines and harmonized protocols for diagnosis and molecular characterisation of agents in animals are very useful and can be used by member countries in establishing animal diagnosis in their countries especially for new and emerging diseases and in improving current diagnostic tests. The regional approach means countries can gain support from other countries in the region and that with the common approach to implementing QA and better diagnostic tests the countries are better able to help each other.

The Regional SOPs and disease algorithms have been used as guidance to establishing country SOPs. The use of these regional guidelines as for influenza and pig diseases along with AAHL SOPs are useful for countries in developing country approaches and should be made available for all key diseases and made available online.

The regional workshops along with the backstopping missions have been very useful in helping to harmonise the diagnosis of disease in the region and have been very useful in capacity building. The back-stopping missions help provide ongoing in-country support.

The laboratory participants and experts discussed testing algorithm for H7N9 ASF, PRRS and CSF for surveillance and investigation and these have been finalised and included in this report (Annex 4, 5, 6, & 7), outlining that for most diseases the primary diagnostic test for disease diagnosis is a specific Real-time PCR, e.g. AI, H7, ASF, PRRS and CSF (Test SOPs are attached to this report) and will be the primary diagnostic test for detection of viral genome. All positive samples can be test further e.g. N9 PCR or a range of positive samples should be referred to a reference laboratory for virus isolation and further characterisation.

Virus isolation was not carrying out in all countries and that conditions for virus isolation varied in each country. It was recognised that laboratory conditions and biosafety and biosecurity needed to be improved in all laboratories carrying out virus isolation, especially for zoonotic agents. It was recommended virus isolation needed to follow OIE guidelines and that there was a need for training at each laboratory on biosafety/biosecurity. Biosafety Class II (BSCII) cabinets must be used for handling diagnostic samples and BSCII were the primary protection for the operator and needed to be tested and calibrated to ensure they are functioning properly. Training in establishing virus isolation capacity for PRRS and CSF in cell culture will be very useful for countries that want to use cell culture.

QA and Biosafety & Biosecurity guidelines are needed for the region and continued support is needed to build capacity in QA and Biosafety. The supply of test SOPs and test paperwork such as coversheets, IQC records and reagent and equipment records have been very useful. The minimum requirement for a diagnostic laboratory for biosafety and quality assurance when handling animal agents were discussed in the workshops and supplied to the participants has been included in this report as attachments.

The workshop participants agreed there were still key capacity gaps in the region for the laboratories to operate to international standard (OIE & ISO17025) which include biosafety and biosecurity, QA, budget and resources. There is a need for all countries to have a QA system in place and as part of that system IQC for tests (standardized reference controls. It is recommended to have regional reference controls that can be used by National laboratories to established National Reference and laboratory reference controls that can be used to confirm laboratories are getting the correct results.

Data collection, sample identification and reporting were discussed and were another area of concern for the laboratories. All laboratories had a system in place to track samples and data in the laboratory but this needed to be improved. There needed to be further support and training in collection and storage of laboratory and field data, participants felt there was not a good connection between the laboratory and the field with both sides mainly working independently of each other.

The training provided by the workshops was very beneficial to the individuals and the laboratories they represent but to gain maximum benefit from the training in this workshop, previous workshops and future workshops, there needs to be commitment from all levels of the animal health system in countries to put in practice the knowledge and techniques learnt. To help this to happen there is a need for funded in-country activities which requires the trainee and the laboratory to use the knowledge and technologies learnt. Surveillance activities should be part of the in-country activity so there is a chance to gain information for the country and a chance to provide data to the country and region for disease control.

ANNEX 1: Daily Agenda of the H7N9 & Pig Diseases Training Workshop



Regional Laboratory Network Training Workshop on Diagnosis and Characterisation of H7N9 Influenza A and Pig Diseases; African Swine Fever (ASF), Classical Swine Fever (CSF) and Porcine Reproductive and Respiratory Syndrome (PRRS)

29th July – 9th August 2013
Regional Animal Health Office No.6
Ho Chi Minh City, Viet Nam
Regional

Workshop Program

Participants will be split into groups for the training. All participants will have hands-on training during the workshop. The workshop will cover Influenza Diagnosis with a focus on H7N9 and pig diseases ASF, CSF & PRRS.

For H7N9 serology participants can bring their own serum samples to test. Training will focus on H7N9 diagnosis and understanding the use of serology and antigen detection tests and the interpretation of the results.

The workshop will review diagnosis of ASF, CSF & PRRS with a focus on ASF. The workshop will cover the establishment of new tests in the laboratory and especially PCR and how to troubleshoot problems and the use of reference controls and current tests in the laboratory to confirm the new test is giving expected sensitivity.

Participants will be given annual Regional Proficiency Test (PT) Panels at the end of the workshop to take back to their laboratories. PT panels will need to be completed on return to the laboratory and laboratories should prepare to complete PT testing by the end of August 2013. All participants need to prepare any paperwork required for them to take reagents (PT samples) back to their laboratory. Paperwork for PT panel will be supplied for customs purposes.

Day 1 Monday 29th July 2013		
0800 – 0830	Registration	RAHO6
0830 – 0900 (R.504)	Opening by DAH, FAO and AAHL	-RAHO6 -DAH -AAHL
	Introduction	All participant
0900 – 0930	Pre-course Questionnaire	All participant
0930--1000	Photo Tea break	
1000 – 1200 (R.504)	Introduction to Workshop <ul style="list-style-type: none"> - Aims - Biosafety - QA & ISO17025 - RAHO - 6 H7N9 overview & Influenza Diagnostic Algorithm	Mr. Chris Morrissy Mr.Long Mr.Chris
1200 – 1300	Lunch	
1300 – 1700 (R.404)	H7N9 Influenza A Serology (Group 1) Haemagglutination Inhibition Test (HI): <ul style="list-style-type: none"> - Titration of reagents H7N9 Influenza A Virus isolation (Group 2) (Use NDV: demonstration) <ul style="list-style-type: none"> - Inoculation of eggs - Biosafety & Class II cabinet demonstration Group 1 and Group 2 change activities	Ms. Julie Mr.Quang Mr.Phuong Mr. Chris Mr.Hung Mr.Dan
Tea break at 1445 – 1515 (R.403 , 409)		
Day 2 Tuesday 30th July 2013		
0800 – 1200	H7N9 Influenza A Serology	Ms. Julie Mr.Quang Mr.Hung
Tea break at 0945- 1015 (R.404)	Testing of serum samples using screening tests. <ul style="list-style-type: none"> - Haemagglutination Inhibition Test (HI): (Group 1) - Type A ELISA (Group 2) 	Mr. Chris Mr.Phuong Mr.Dan
1200 – 1300	Lunch	

1300 – 1700	H7N9 Influenza A Serology	
Tea break at 1445 – 1515 (R.404)	Testing of serum samples using screening tests. – Haemagglutination Inhibition Test (HI): (Group 2) – Type A ELISA (Group 1)	Ms. Julie Mr.Quang Mr.Hung Mr. Chris Mr.Phuong Mr.Dan
Day 3 Wednesday 31st. July 2013		
0800 – 1200 (R.404)	H7N9 Influenza A Serology	
Tea break at 0945- 1015	Haemagglutination Inhibition Test (HI): – Titration of positive serum. (Group 1) – Interpretation & Analysis of results H7N9 Influenza A PCR: (Group 2) – Preparation of Primers & Probe – Extraction of samples H7N9 Influenza A Virus isolation Candle eggs	Ms. Julie Mr.Phuong Mr.Dan Mr. Frank Mr. Chris Mr.Vu Mr.Quang Mr.Chris Mr.Hung
1200 – 1300	Lunch	
1300 – 1700 (R.404)	H7N9 Influenza A Serology (Group 2)	Ms. Julie Mr.Phuong
Tea break at 1445 – 1515	H7N9 Influenza A PCR: (Group 1) H7N9 Influenza A Virus isolation Candle eggs	Mr. Frank Mr.Vu Mr.Quang Mr.Chris Mr.Hung
Day 4 Thursday 01st. August 2013		
0800 – 1200 (R.404)	H7N9 Influenza A PCR (Group 1) – Test samples using Type A, H7 & N9 PCR Tests	Mr. Frank Mr.Vu Mr.Quang
Tea break at 0945- 1015 (R.403,409)	H7N9 Influenza A Virus isolation (Group 2) – Harvest Eggs – Candle eggs	Ms.Julie Mr.Chris Mr.Hung Mr.Dan

		Mr.Phuong
1200 – 1300	Lunch	
1300 – 1700 (R.404)	H7N9 Influenza A PCR (Group 2)	Mr. Frank Mr. Vu Mr. Quang
Tea break at 1445 - 1515	H7N9 Influenza A Virus isolation (Group 1)	Ms. Julie Mr. Chris Mr. Hung, Mr. Dan Mr. Phuong Mr. Chris
	QA & ISO17025	
Day 5 Friday 02nd. August 2013		
0800 – 1200 (R.404)	H7N9 Influenza A PCR	Mr. Frank Mr. Vu Mr. Quang
Tea break at 0945- 1015	<ul style="list-style-type: none"> - Report results with recommendations - Interpretation & Analysis of results - Review QA and IQC - Discussion on trouble shooting 	
(R.409)	H7N9 Influenza A Virus isolation Candle & Harvest eggs	Ms. Julie Mr. Chris Mr. Hung, Mr. Dan Mr. Phuong
1200 – 1300	Lunch	
1300 – 1700 (R.409)	H7N9 Influenza A Virus isolation	Ms. Julie Mr. Chris Mr. Hung, Mr. Dan Mr. Phuong
Tea break at 1445 - 1515	- Candle & Harvest eggs	
	Review of Pig Diseases ASF, CSF & PRRS	
Day 6 Monday 05th. August 2013		
0800 – 1200 (R.404)	H7N9 Influenza A & ASF PCR	Mr. Frank Mr. Chris Mr. Vu Mr. Quang Mr. Phuong
Tea break at 0945- 1015	Extraction of samples Diagnostic Pathology for Influenza and Pig Diseases.	
1200 – 1300	Lunch	
1300 – 1700 (R.404)	H7N9 Influenza A PCR	Mr. Frank Mr. Chris

Tea break at 1445 - 1515	Test VI samples using Type A H7 & N9 PCR Tests	Mr. Vu Mr. Quang Mr. Phuong Mr. Hung
Day 7 Tuesday 06th. August 2013		
0800 – 1200 (R.404)	Identification of VI sample (H7N9) by HA Using Reference serum	Mr. Chris Mr. Phuong Mr. Hung
Tea break at 0945- 1015	ASF PCR SOP for establishing a new test in your laboratory Use of QA and IQC reference controls	Mr. Frank Mr. Chris Mr. Vu Mr. Quang
1200 – 1300	Lunch	
1300 – 1700 (R.404)	Identification of VI sample (H7N9) by HA Using Reference serum	Mr. Chris Mr. Phuong Mr. Hung
Tea break at 1445 - 1515	ASF PCR SOP for establishing a new test in your laboratory Use of QA and IQC reference controls	Mr. Frank Mr. Chris Mr. Vu Mr. Quang
Day 8 Wednesday 08th. August 2013		
0800 – 1200 (R.404)	H7N9 Influenza A PCR Interpretation & Analysis of results from VI samples	Mr. Frank Mr. Chris Mr. Vu Mr. Quang Mr. Phuong Mr. Hung
Tea break at 0945- 1015	Review QA and IQC Report results with recommendations Discussion on trouble shooting	
1200 – 1300	Lunch	
1300 – 1700 Tea break at 1445 - 1515	ASF PCR Interpretation & Analysis of result Review QA and IQC	Mr. Frank Mr. Chris Mr. Vu Mr. Quang Mr. Phuong Mr. Hung

	Report results with recommendations Discussion on trouble shooting Sequencing & Bioinformatics for Influenza	
Day 9 Thursday 08th. August 2013		
0800 – 1200 (R.504) Tea break at 0945- 1015	ASF & H7N9 PCR Further tests set-up SOP for Establishing a new test in your laboratory Agree on SOP Reporting results Discuss Problems	Mr. Frank Mr. Chris Mr. Vu Mr. Quang Mr. Phuong Mr. Hung
1200 – 1300	Lunch	
1300 – 1700 (R.504) Tea break at 1445 - 1515	ASF & H7N9 PCR SOP for Establishing a new test in your laboratory Using Network and reference laboratories Discussions with Epidemiology grp for use of new test and sensitivity and specificity required.	Mr. Frank Mr. Chris Mr. Vu Mr. Quang Mr. Phuong Mr. Hung
Day 10 Friday 09th. August 2013		
0800 – 0945 (R.504) Tea break at 0945- 1015 1015 - 1200	Results Discussion Post-course Questionnaire Electronic Copies of Methods, results Introduction and demonstration on PPE by 3M company	Mr. Chris Mr. Frank 3M company
1200 – 1300	Lunch	
1300 – 1400 (R.504) 1400-1500	Proficiency Panel delivery Instruction on testing required and due date Conclusion and closing	Mr. Chris Mr. Phuong Mr. Hung Mr. Quang FAO, DAH, AAHL, RAHO6

Remark: TEA BREAK AND LUNCH AT ROOM 503

Things needed for HI HA for serology & virology and PCR.

20 Participants so 10 teams in 2 groups (work in pairs).

AAHL

1. HI reagents & controls H5, H7 (x2) & H9. Also NDV. For serology & Identification.
Reference sera H5, H7, H9.
2. Type A ELISA reagents

RAHO- 6

1. Buffers & RBC's for HI HA
2. 8 – 10 serum samples for HI testing. H5 positive (2) H7 positive (3) H9 positive (2) Unknown (3).
Note : these to be tested in ELISA as well. We most likely need 5 ml of each.
3. 20 Type A positive serum samples and 10 negative serum samples (that are mainly ducks maybe ducks give more H positives) to add to 8 – 10 sera above
4. NDV high & low path isolates
5. RAHO-6 need to list other reagents, PCR kits buffers & plastic ware needed

ANNEX 2: Participants list of the ASF, CSF and PRRS Training Workshop



USAID
FROM THE AMERICAN PEOPLE



Regional Laboratory Network Workshop on Diagnosis and Characterisation of Influenza A (H7N9) and Priority Swine Diseases; African Swine Fever (ASF), Classical Swine Fever (CSF) and Porcine Reproductive and Respiratory Syndrome (PRRS)

29 July - 9 August 2013
Regional Animal Health Office No.6
Ho Chi Minh City, Viet Nam

Email: lilikprayitno58@gmail.com

Cambodia

Ms. Ren Theary

Head of Virology/Serology
National Veterinary Research Institute
Department of Animal Production and Health
Trea village, str. 371,
Sangkat Steung Mean Chey, Khan Mean Chey,
Phnom Penh, Cambodia
Tel: +855 97 3435408
Fax: +855 23 969 019
Mobile: +855 97 3435408
Email: rentheary@ymail.com

Ms. Seng Bunnary

Staff of Virology/Serology
National Veterinary Research Institute
Department of Animal Production and Health
Trea village, Street 371,
Sangkat Steung Mean Chey, Khan Mean Chey,
Phnom Penh, Cambodia
Tel: +855 16 867 121
Fax: +855 23 969 019
Mobile: +855 16 867 121
Email: bunnarym@yahoo.com

Indonesia

Mr. Lilik Prayitno

Staff of Virology Laboratory
Disease Investigation Center (DIC) Medan,
Directorate General Livestock and Animal
Health Services
Jenderal Gatot Subroto Street No.255A,
Medan 20127 Indonesia
Tel: +6261-8452253
Fax: +6261-8469911
Mobile: +6281396749759

Mr. Sunarno

Coordinator of molecular biology laboratory
Disease Investigation Center (DIC) Subang,
Directorate General Livestock and Animal
Health Services

Jl. Terusan Garuda Blok Werasari RT 33/RW
11, Dangdeur, Subang, Jawa Barat 41212
Indonesia
Tel: +6260 7423134
Fax: +6260 7423178
Mobile: +6285624863557, +6285317689775
Email: ano_dvm@yahoo.com;
ano.keswan@gmail.com

Lao PDR

Ms. Vilayvanh Soukvilay

Senior Technical Laboratory Staff
National Animal Health Laboratory
Department of Livestock and Fisheries
Sithane Neua village, Sikhottabong district,
Vientiane Capital, Lao PDR.
Tel: +856-21-216380
Fax: +856-21-216380
Mobile: +856-20-55204045
Email: VL.soukvilay@yahoo.com

Mr. Khamkhoun Soundala

Technical Laboratory Staff
National Animal Health Laboratory
Department of Livestock and Fisheries
Sithane Neua village, Sikhottabong district,
Vientiane Capital, Lao PDR.
Tel: +856-21-216380
Fax: +856-21-216380
Mobile: +856-20-22080209

Email: VL.soukvilay@yahoo.com

Visayas Avenue, Diliman
Quezon City, 1100 Philippines
Tel: +63 2 928-2177, +63 2 920-0429
Fax: +63 2 920-0429
Mobile: +63 922-8010418
Email: cflegaspi@yahoo.com

Malaysia

Mr. Muhammad Redzwan bin Sidik
Research Officer
Veterinary Research Institute
Department of Veterinary Services
59, Jalan Sultan Azlan Shah,
31400 Ipoh, Perak, Malaysia
Tel: +605 5457166
Fax: +605 5463368
Mobile: +6017 6534075
Email: tun_melaka@yahoo.com

Ms. Rafidah binti Ahmad Johari
Laboratory Technician
Regional Veterinary Laboratory Bukit Tengah
P.O. Box 63
14007 Bukit Tengah, Bukit Mertajam,
Penang, Malaysia
Tel: +604 5072540
Fax: +604 5075796
Mobile: +6019 5479690
Email: rjh.1106@yahoo.com

Myanmar

Mr. Maung Maung Soe
Research Officer
Mandalay Diagnostic Laboratory
Livestock Breeding and Veterinary Department
Mandalay, Myanmar
Tel: +95-67-408262
Fax: +95-67-408342, 408044
Mobile: +95-9-2010637
Email: lbvd@mptmail.net.mm;
ytwvet84@gmail.com

Mr. Aung Thu
Researcher
Yangon Diagnostic Laboratory
Livestock Breeding and Veterinary Department
Yangon, Myanmar
Tel: +95-67-408262
Fax: +95-67-408342, 408044
Mobile: +95-9-420149201
Email: lbvd@mptmail.net.mm;
aungaungthu1986@gmail.com

Philippines

Ms. Cristina Legaspi
Senior Agriculturist
Philippine Animal Health Center (PAHC)
Bureau of Animal Industry (BAI)

Ms. Edna A. Felipe
Senior Agriculturist
Philippine Animal Health Center (PAHC)
Bureau of Animal Industry (BAI)
Visayas Avenue, Diliman
Quezon City, 1101 Philippines
Tel: +63 2 9282177
Fax: +63 2 9282177
Mobile: +639178349192
Email: edna5200@yahoo.com

Thailand

Mr. Prakrit Boonpornprasert
Veterinary Officer
National Institute of Animal Health
50/2 Moo 3 Phaholyothin Rd.,
Ladysao, Chatuchak,
Bangkok 10900 Thailand
Tel: +662 579 8908 to 14 ext. 424
Fax: +662 579 8918 to 19
Mobile: +6684 003 1133
Email: pkbpps@hotmail.com

Mr. Bopit Puyati
Veterinary Officer
Veterinary Research and Development Center
(Lower Northeastern Region),
Surin Province, Thailand
Tel: +66 44 546 104
Fax: +66 44 546 147
Mobile: +6689 809 5804
Email: boybopit@hotmail.com

Viet Nam

Ms. Do Thi Hoa
Veterinarian
National Center for Veterinary Diagnostics,
Department of Animal Health
78/11 Giai Phong, Phuong Mai, Dong Da,
Hanoi, Viet Nam
Tel: +84 4 3869 1151
Fax: +84 4 3868 6813
Mobile: +84 1686954956
Email: hoancvd@gmail.com

Ms. Nguyen Thi Trang
Technician
National Center for Veterinary Diagnostics,
Department of Animal Health
78/11 Giai Phong, Phuong Mai, Dong Da,

Hanoi, Viet Nam
Tel: +84 4 3869 1151
Fax: +84 4 3868 6813
Mobile: +84 942945252
Email: trangncvd@gmail.com

Mr. Le Tri Dang

Virology Section staff
Regional Animal Health Office No.6
521/1 Hoang Van Thu St., Ward 4,
Tan Binh District,
Ho Chi Minh City, Vietnam
Tel: +84 8 3948 3034
Fax: +84 8 3948 3031
Mobile: + 84 982356631
Email: Letridang86@gmail.com

Mr. Tran Minh Tan

Virology Section staff
Regional Animal Health Office No.6
521/1 Hoang Van Thu St., Ward 4,
Tan Binh District,
Ho Chi Minh City, Vietnam
Tel: +84 8 3948 3044
Fax: +84 8 3948 3031
Mobile: +84 932 747 828
Email: Tranminhtan06@gmail.com

**Australian Animal Health Laboratory
CSIRO Livestock Industries
(Trainers)**

Dr. Chris Morrissy

Scientific Coordinator AAHL Regional Program
Project Leader Capacity Building
Australian Animal Health Laboratory
PMB 24 Geelong 3213, Australia
Tel: +61 3 5227 5000
Fax: +61 3 52275555
Mobile: +61 0 419145504
Email: chris.morrissy@csiro.au

Dr. Frank Wong

Research Scientist
Diagnosis, Surveillance & Response (DSR)
Australian Animal Health Laboratory
CSIRO Livestock Industries
PMB 24 Geelong 3213, Australia
Tel: +61 3 5227 5000
Fax: +61 3 5227 5555
Email: frank.wong@csiro.au

Dr. Julie Cooke

Diagnostic Virologist
Diagnosis, Surveillance & Response (DSR)
CSIRO Australian Animal Health Laboratory
PMB 24 Geelong 3213
Australia
Tel: +61 3 5227 5000
Fax: +61 3 5227 5555
Email: julie.cooke@csiro.au

**Regional Animal Health Office No.6
Department of Animal Health
(Trainer's Assistant)**

Dr. Ngo Thanh Long

Center for Veterinary Diagnostics
Regional Animal Health Office No.6
521/1 Hoang Van Thu, Ward 4, Tan Binh Dist.,
Ho Chi Minh City, Vietnam
Tel: +84 8 39483034
Fax: +84 8 39483031
Mobile: +84 913 894 891
Email: ngothanhlong60@gmail.com

Dr. Pham Phong Vu

Head of Virology Section
Deputy Manager of Center for Veterinary
Diagnostics
Regional Animal Health Office No.6
521/1 Hoang Van Thu St., Ward 4, Tan Binh
Dist., Ho Chi Minh City, Viet Nam
Phone: (+84) 8 39483044
Cell phone: (+84) 903914464
Fax: (+84) 8 39483031
Email: phamphongvu@gmail.com

Dr. Vo Van Hung

Laboratory Staff
Center for Veterinary Diagnostics
Regional Animal Health Office No. 6
521/1 Hoang Van Thu Str., Ward 4, Tan Binh
Dist., Ho Chi Minh City, Viet Nam.
Tel: + 84 8 3948 3034
Fax: + 84 8 3948 3031
Mobile: + 84 976861363
Email: vovanhung.raho6@gmail.com;
vvhdhnl@yahoo.com

Dr. Le Thi Quynh Anh

Laboratory Staff
Center for Veterinary Diagnostics
Regional Animal Health Office No. 6
521/1 Hoang Van Thu Str., Ward 4, Tan Binh
Dist., Ho Chi Minh City, Viet Nam.
Tel: +84 8 39483044
Fax: +84 8 39483031
Mobile: +84 916172346
Email: quynhanh.raho6@gmail.com

DAH-Vietnam

Dr. (Mr) Mai Van Hiep

Deputy Director General
Department of Animal Health (DAH) rep unit in
HCM
Ministry of Agriculture and Rural Development
24 Hoang Van Thu, Tan Binh, Ho Chi Minh
Tel: +84-8-3845-2528
Fax:+84-8-3844-4029
E-mail: hiepmaidah@gmail.com

Annex 3: Weekly Agenda of the H7N9 & Pig Diseases Training Workshop

Regional Laboratory Network Training Workshop on Diagnosis and Characterisation of H7N9 Influenza A and Pig Diseases; African Swine Fever (ASF), Classical Swine Fever (CSF) and Porcine Reproductive and Respiratory Syndrome (PRRS)

**29th July – 9th August 2013
Regional Animal Health Office No.6
Ho Chi Minh City, Viet Nam
Regional**

Workshop Program

Participants will be split into groups for the training. All participants will have hands-on training during the workshop. The workshop will cover Influenza Diagnosis with a focus on H7N9 and pig diseases ASF, CSF & PRRS.

For H7N9 serology participants can bring their own serum samples to test. Training will focus on H7N9 diagnosis and understanding the use of serology and antigen detection tests and the interpretation of the results.

The workshop will review diagnosis of ASF, CSF & PRRS with a focus on ASF. The workshop will cover the establishment of new tests in the laboratory and especially PCR and how to troubleshoot problems and the use of reference controls and current tests in the laboratory to confirm the new test is giving expected sensitivity.

Participants will be given annual Regional Proficiency Test (PT) Panels at the end of the workshop to take back to their laboratories. PT panels will need to be completed on return to the laboratory and laboratories should prepare to complete PT testing by the end of August 2013. All participants need to prepare any paperwork required for them to take reagents (PT samples) back to their laboratory. Paperwork for PT panel will be supplied for customs purposes.

WEEK ONE SCHEDULE

TIME	Monday	Tuesday	Wednesday	Thursday	Friday
8.00 – 12.30	<p>Welcome & Introduction.</p> <p>Pre-course Questionnaire</p> <p>Introduction to Workshop</p> <ul style="list-style-type: none"> - Aims - RAHO - 6 - Biosafety - QA & ISO17025 <p>H7N9 overview & Influenza Diagnostic Algorithm</p>	<p>H7N9 Influenza A Serology</p> <p>Testing of serum samples using screening tests.</p> <ul style="list-style-type: none"> - Haemagglutination Inhibition Test (HI): (Group 1) <ul style="list-style-type: none"> o 40 samples H7 & H5 - Type A ELISA (Group 2) <ul style="list-style-type: none"> o 40 samples <p>H7N9 Influenza A Virus isolation</p> <ul style="list-style-type: none"> - Candle eggs 	<p>H7N9 Influenza A Serology</p> <p>Haemagglutination Inhibition Test (HI):</p> <ul style="list-style-type: none"> - Titration of positive serum. (Group 1) - Interpretation & Analysis of results <p>H7N9 Influenza A PCR: (Group 2)</p> <ul style="list-style-type: none"> - Preparation of Primers & Probe - Extraction of samples <p>H7N9 Influenza A Virus isolation</p> <ul style="list-style-type: none"> - Candle eggs 	<p>H7N9 Influenza A PCR (Group 1)</p> <ul style="list-style-type: none"> - Test samples using Type A, H7 & N9 PCR Tests <p>H7N9 Influenza A Virus isolation (Group 2)</p> <ul style="list-style-type: none"> - Harvest Eggs - Candle eggs 	<p>H7N9 Influenza A PCR H7N9 Influenza A Serology</p> <ul style="list-style-type: none"> - Report results with recommendations - Interpretation & Analysis of results - Review QA and IQC - Discussion on trouble shooting <p>H7N9 Influenza A Virus isolation</p> <ul style="list-style-type: none"> - Candle & Harvest eggs
1.30 – 4.30	<p>H7N9 Influenza A Serology (Group 1)</p> <p>Haemagglutination Inhibition Test (HI):</p> <ul style="list-style-type: none"> - Titration of reagents <p>H7N9 Influenza A Virus isolation (Group 2) (Use NDV: demonstration)</p> <ul style="list-style-type: none"> - Inoculation of eggs - Biosafety & Class II cabinet demonstration 	<p>H7N9 Influenza A Serology</p> <p>Testing of serum samples using screening tests.</p> <ul style="list-style-type: none"> - Haemagglutination Inhibition Test (HI): (Group 2) - Type A ELISA (Group 1) 	<p>H7N9 Influenza A Serology (Group 2)</p> <p>H7N9 Influenza A PCR: (Group 1)</p> <p>H7N9 Influenza A Virus isolation</p> <p>Candle eggs</p>	<p>H7N9 Influenza A PCR (Group 2)</p> <p>H7N9 Influenza A Virus isolation (Group 1)</p> <p>QA & ISO17025</p>	<p>H7N9 Influenza A Virus isolation</p> <ul style="list-style-type: none"> - Candle & Harvest eggs <p>Review of Pig Diseases ASF, CSF & PRRS</p>

WEEK TWO SCHEDULE

TIME	Monday	Tuesday	Wednesday	Thursday	Friday
8.00 – 12.30	<p>ASF PCR (Group 1)</p> <ul style="list-style-type: none"> Preparation of Primers & Probe Extraction of samples <p>Identification of VI sample (H7N9) by HA/HI (Group 2)</p> <ul style="list-style-type: none"> Using Reference serum <ul style="list-style-type: none"> H7, H5 & H9 	<p>ASF PCR & Influenza H Typing (Group 1)</p> <ul style="list-style-type: none"> SOP for establishing a new PCR test in your laboratory Use of QA and IQC reference controls Sensitivity & specificity Titration of Positive Control <p>Laboratory Workflow & Diagnosis (Group 2)</p> <ul style="list-style-type: none"> Pig Diseases Cell Culture VI & Identification Pathology 	<p>H Typing</p> <ul style="list-style-type: none"> Run Gel <p>ASF , H7N9 Influenza A PCR & H Typing</p> <p>Identification of VI sample (H7N9) by HA/HI</p> <ul style="list-style-type: none"> Interpretation & Analysis of results from VI samples Review QA and IQC Report results with recommendations Discussion on trouble shooting 	<p>ASF & H7N9 PCR</p> <p>Group Discuss</p> <ul style="list-style-type: none"> Regional SOP for Establishing a new test in your laboratory Agree on PCR SOP Reporting results Discuss Troubleshooting <p>Recommendations & Conclusions</p>	<p>Discussion</p> <ul style="list-style-type: none"> Electronic copy of workshop information <p>Post-course Questionnaire</p> <p>Biosafety: Discussion and Demonstration on PPE for laboratory and field</p>
1.30 – 4.30	<p>ASF PCR (Group 2)</p> <ul style="list-style-type: none"> Preparation of Primers & Probe Extraction of samples <p>Identification of VI sample (H7N9) by HA/HI (Group 1)</p> <ul style="list-style-type: none"> Using Reference serum 	<p>ASF PCR & Influenza H Typing (Group 1)</p> <p>Laboratory Workflow & Diagnosis (Group 2)</p>	<p>Sequencing & Bioinformatics for Influenza</p> <ul style="list-style-type: none"> H & N typing SOP Software Analysis & Interpretation 	<p>ASF & H7N9 PCR</p> <ul style="list-style-type: none"> Regional SOP for Establishing a new test in your laboratory Using Network and reference laboratories Discussions with Epidemiology grp for use of new test and sensitivity and specificity required. 	<p>Proficiency Panel delivery Instruction on testing required and due date</p> <p>Conclusion & Certificates</p>

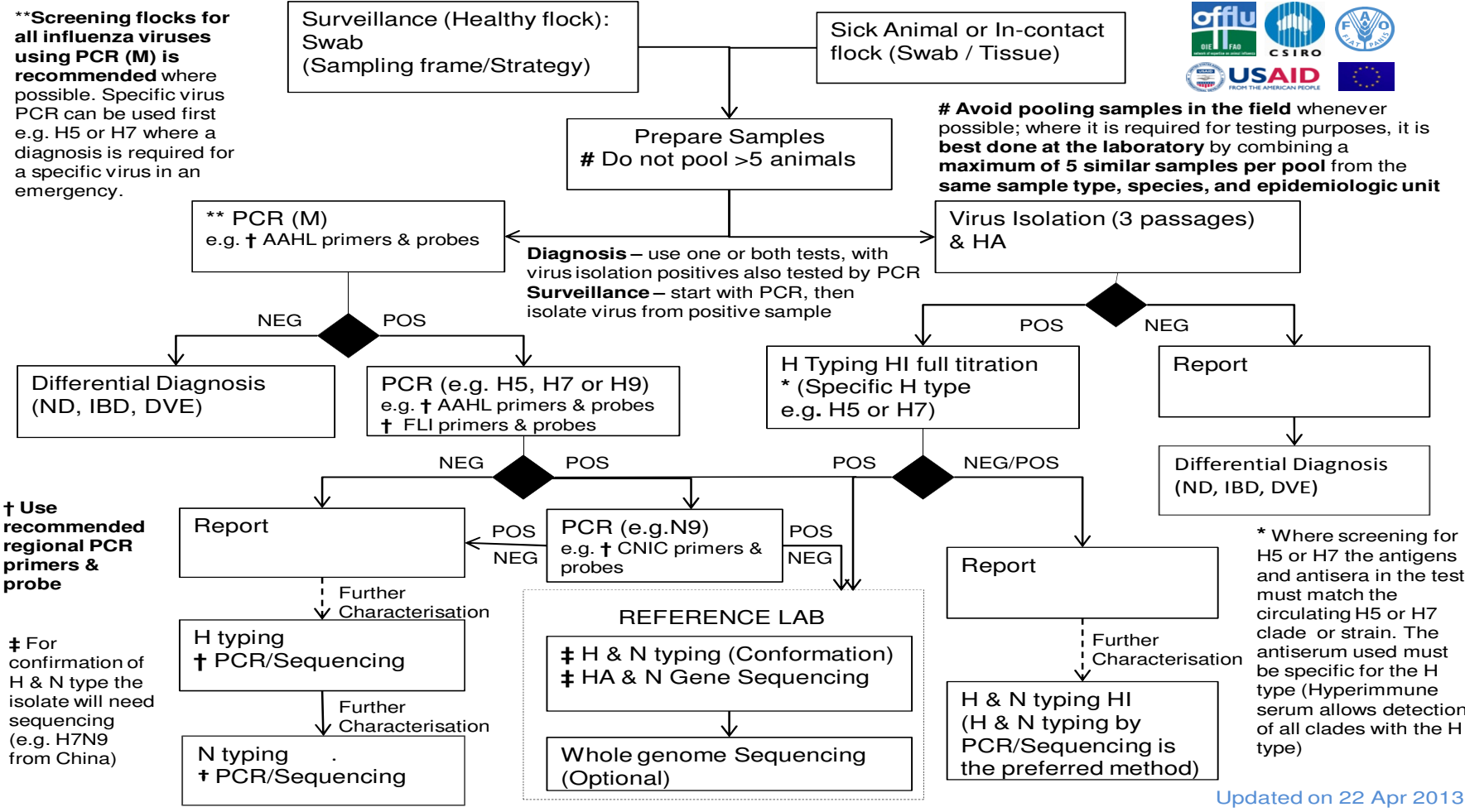
ANNEX 4: SEA Regional Laboratory Network: Laboratory Diagnostic Test Algorithms for Avian Influenza A Surveillance and Investigation

- This update of the existing laboratory algorithms for detection of influenza virus in specimens and for serology
- The preferred comprehensive strategy is to test both surveillance and diagnostic specimens with an approach that will detect any influenza virus infection and then characterize that infection with respect to both H type and N type
- The new algorithm included in this advice outlines an approach for testing of surveillance and diagnostic specimens for evidence of specific H and N type (e.g.H5 or H7), in particular H7 & N9 or H7N9 infection, a targeted approach in the current situation specifically to detect or rule out avian influenza A(H7N9) infection in sampled flocks and birds
- Laboratory testing should be done in accordance with the updated Regional SOPs and guidelines, that now include tests for the detection of avian influenza A(H7N9) infections

Avian Influenza Investigation: Laboratory Algorithm



****Screening flocks for all influenza viruses using PCR (M) is recommended** where possible. Specific virus PCR can be used first e.g. H5 or H7 where a diagnosis is required for a specific virus in an emergency.

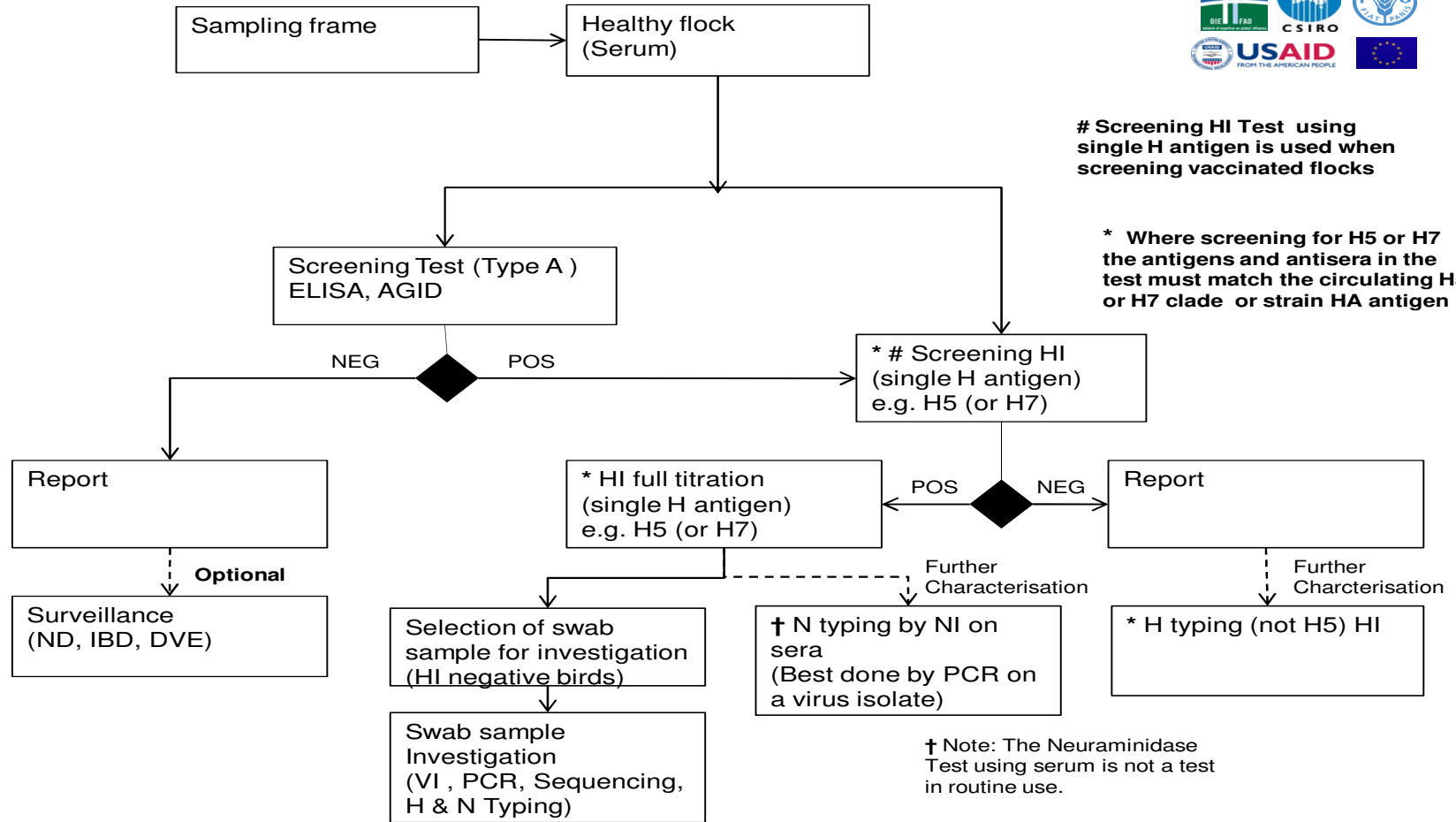


† Use recommended regional PCR primers & probe

‡ For confirmation of H & N type the isolate will need sequencing (e.g. H7N9 from China)

Updated on 22 Apr 2013

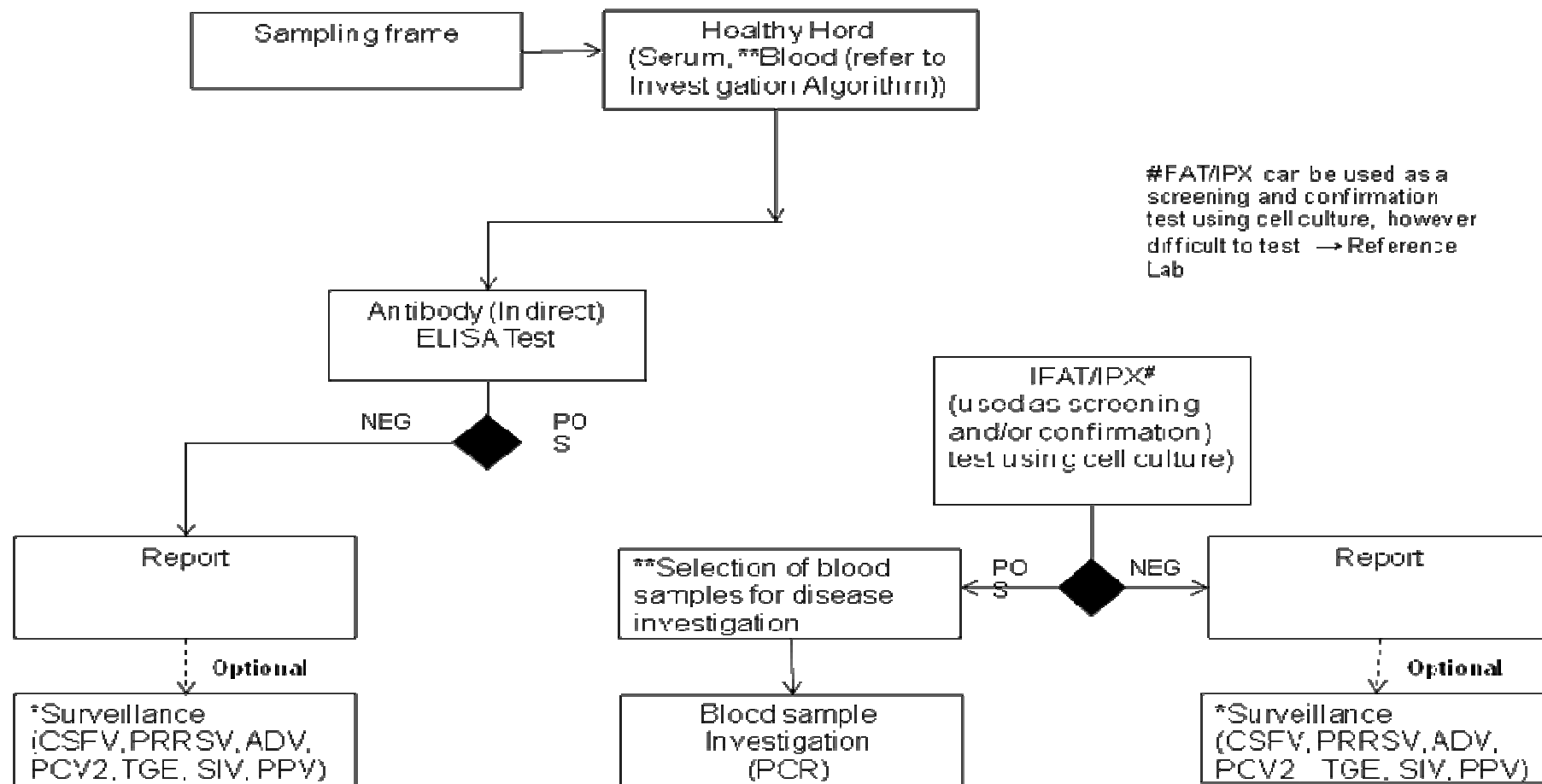
Avian Influenza Surveillance Laboratory Algorithm



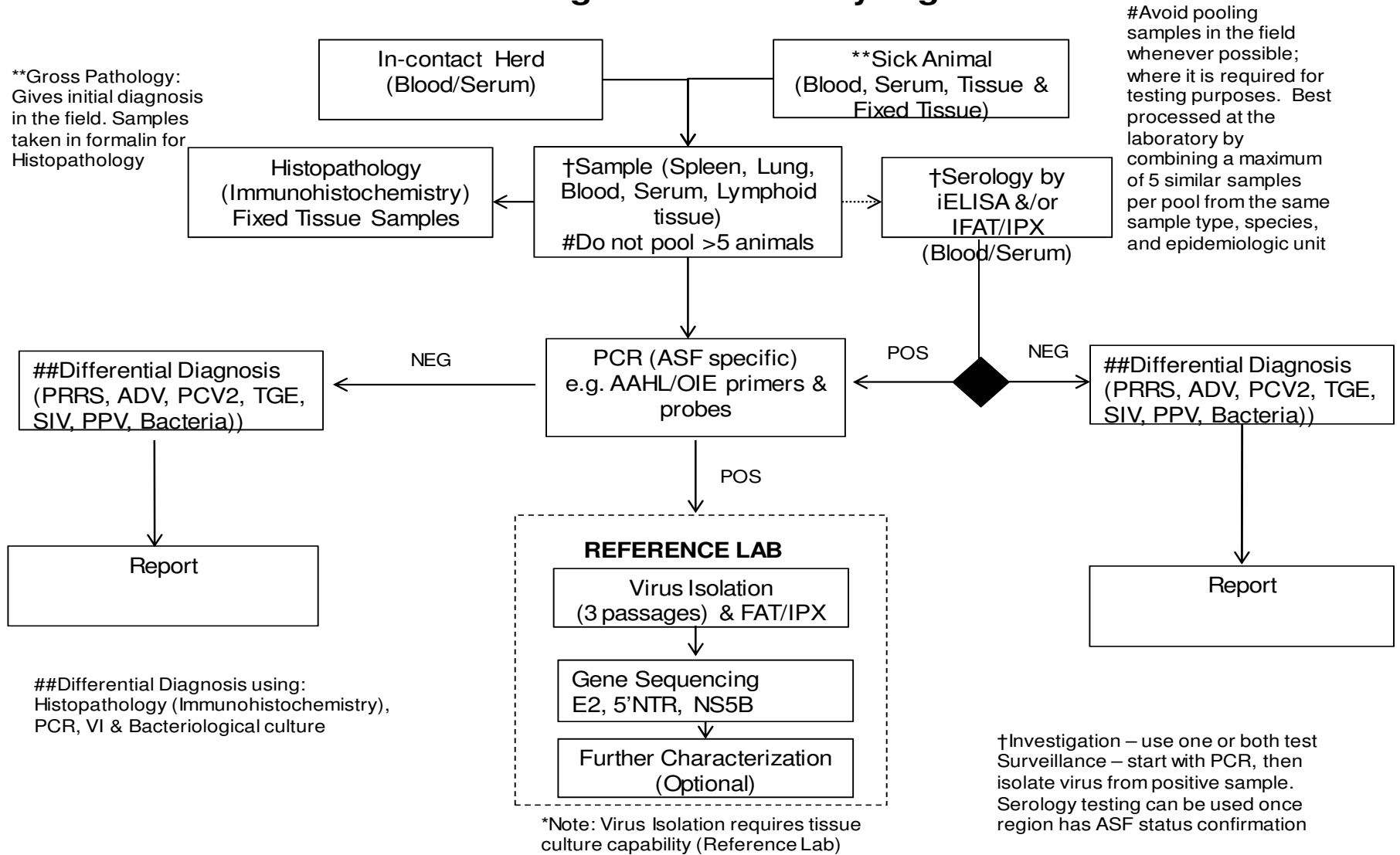
Updated on 22 Apr 2013

ANNEX 5: ASF Diagnostic Test Algorithm for Surveillance & Investigation

African Swine Fever Surveillance Laboratory Algorithm

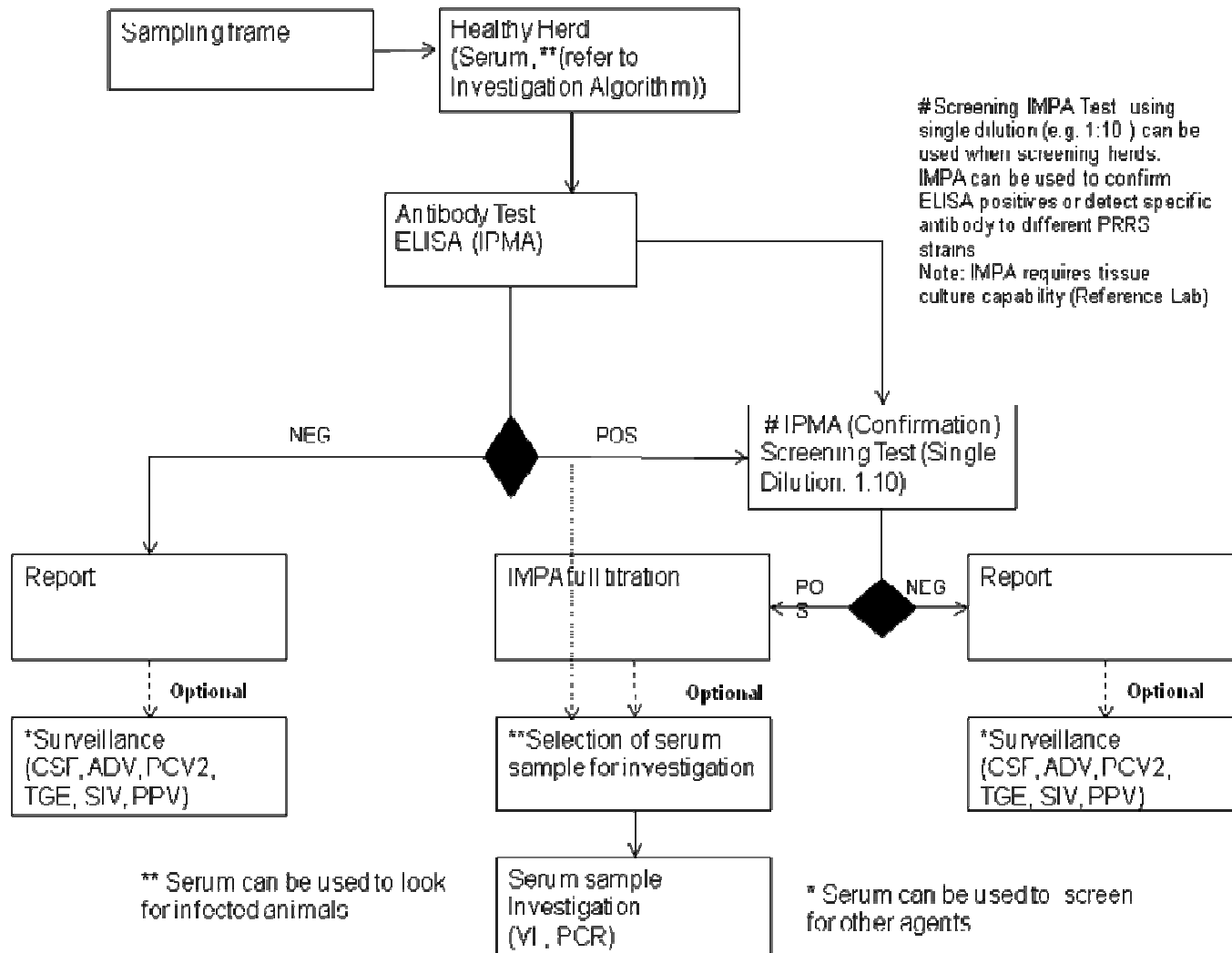


ASF Investigation Laboratory Algorithm

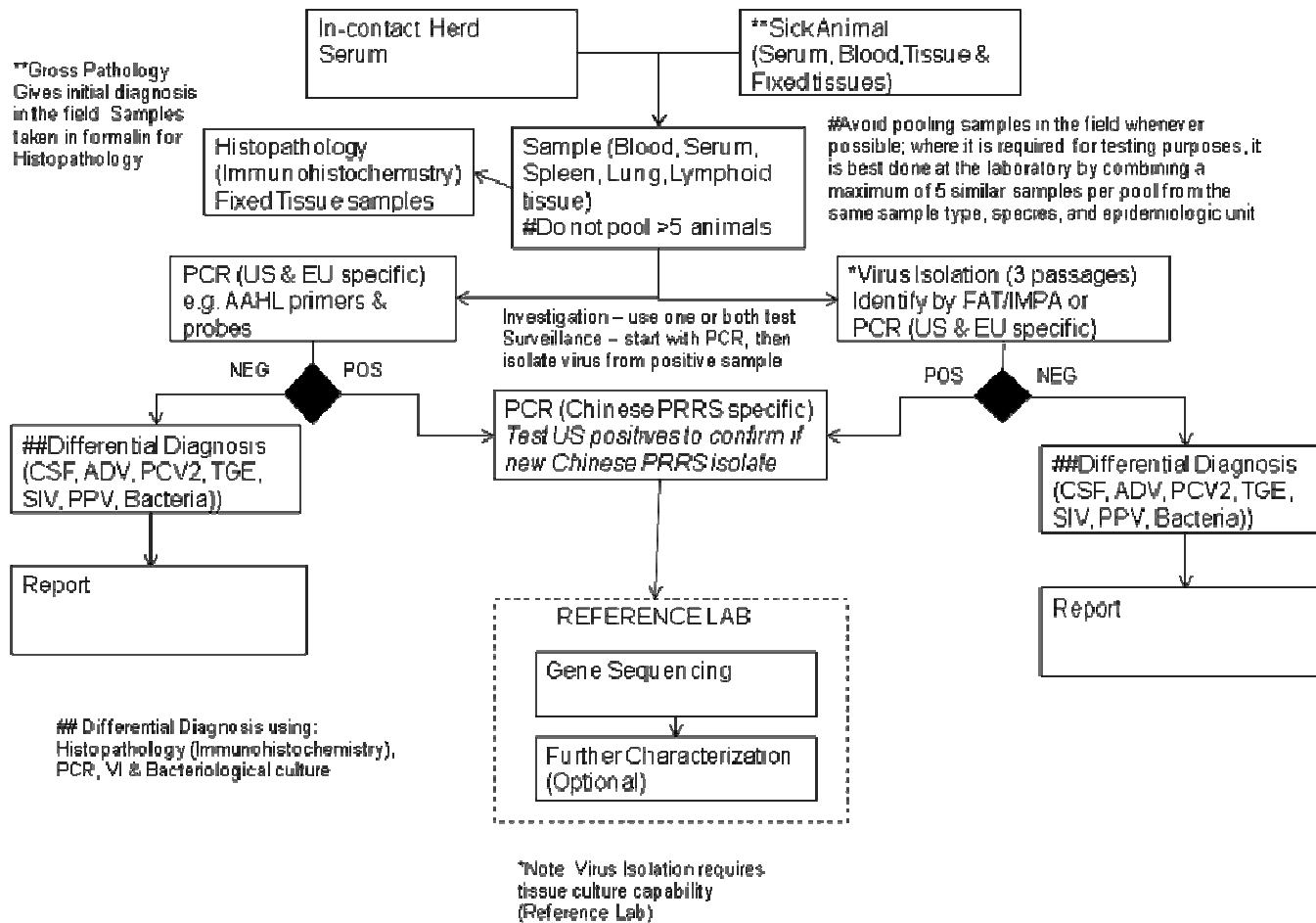


ANNEX 6: PRRS Diagnostic Test Algorithm for Surveillance & Investigation

PRRS Surveillance Laboratory Algorithm

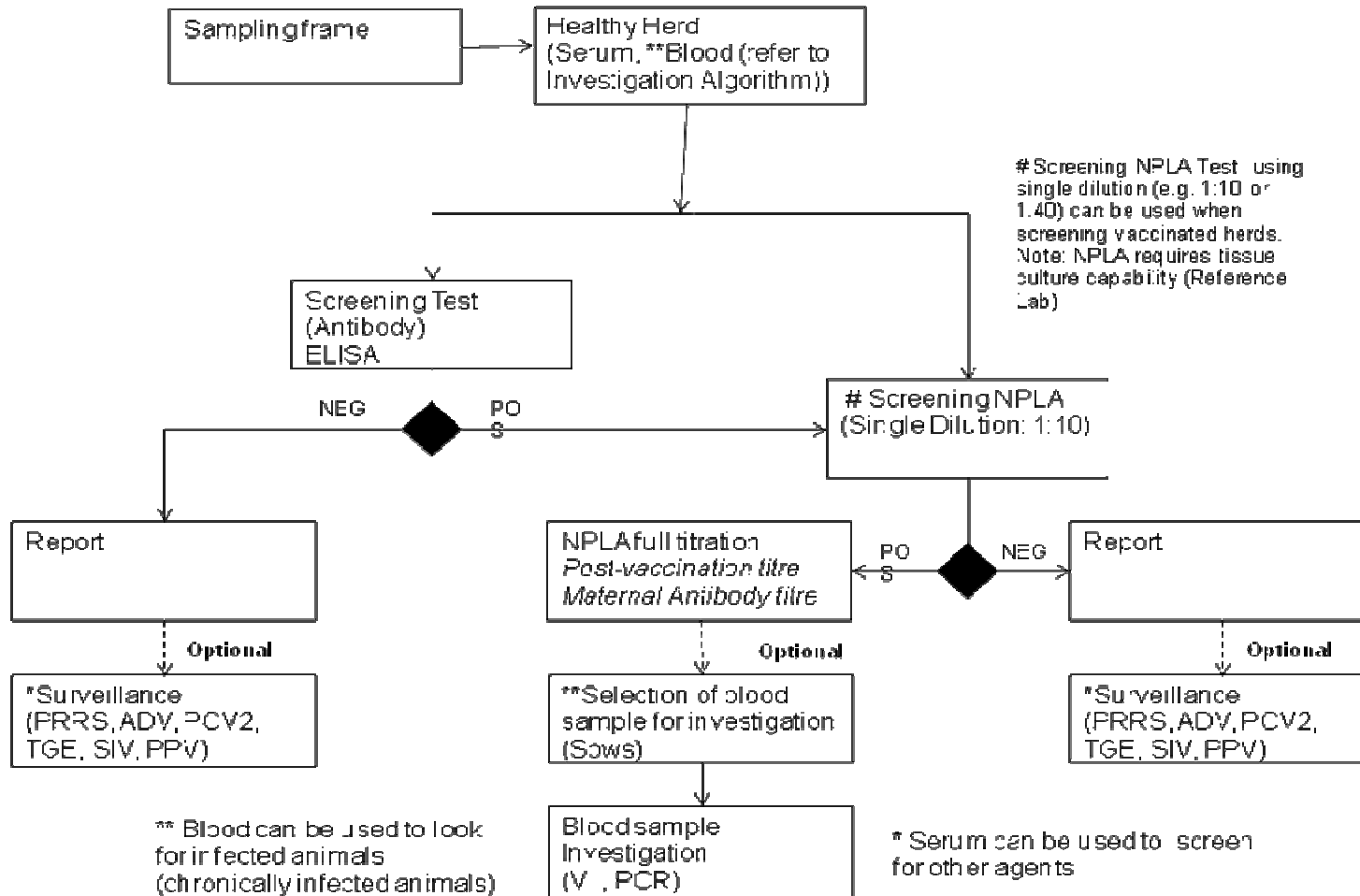


PRRS Investigation Laboratory Algorithm

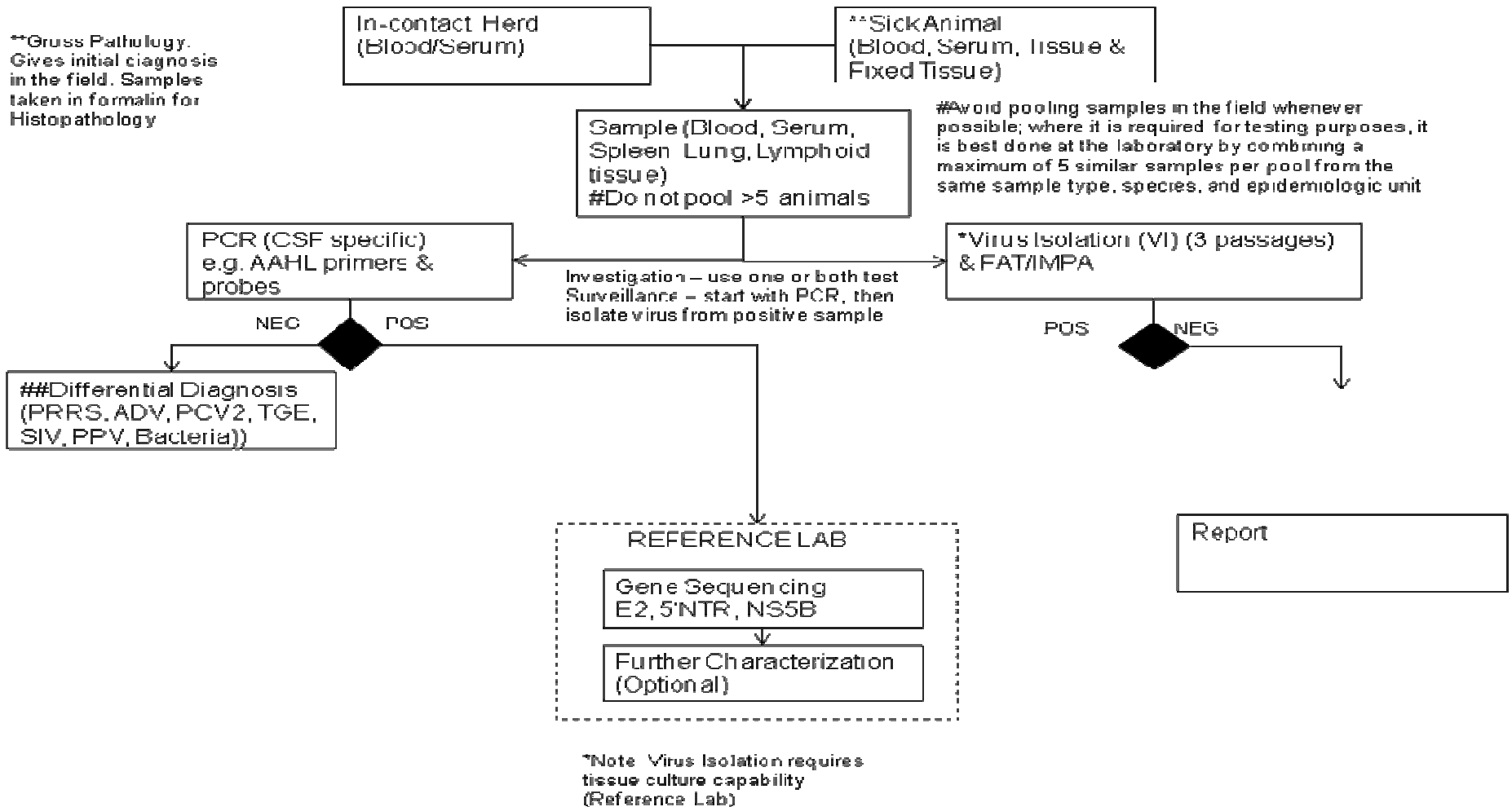


ANNEX 7: CSF Diagnostic Test Algorithm for Surveillance & Investigation

Classical Swine Fever Surveillance Laboratory Algorithm



CSF Investigation Laboratory Algorithm



Annex 8: Pre/Post Training Workshop Laboratory Questionnaire

**Questionnaire for participants attending the H7N9 and Pig Diseases workshop in
Ho Chi Minh City, Vietnam**

1. What temperature and time duration should you heat inactivate diagnostic serum samples before testing in the laboratory?
 - Temperature:
 - Time:

2. What control standards should be included in a Flu-bElisa test?

3. Why is a progressive monitoring record needed for diagnostic test results?

4. What control standards should be included in a HI test?

5. What control standards should be included in a PCR test?

6. List 5 types of equipment that should be calibrated on a regular basis in the laboratory?

-
-
-
-
-

7. What purpose does the adsorption of sera with 10% CRBC play in the HI test?

8. What conclusion can be drawn when a sample gives a positive Elisa result but a negative HI result?

9. What is the difference between a laminar flow cabinet and a biological safety cabinet?

10. Is there cross-reactivity between subtypes of avian influenza? Give an example

11. Does the Flu-Elisa or HI give us any information about the vaccination status of particular sera?

12. Which of the following is TRUE (T) when referring to a TaqMan real-time PCR reaction?

- a. Need to use fluorescently labelled Forward Primer and Reverse Primer T / F
- b. The progress of the PCR reaction can be monitored from START to FINISH T / F
- c. Need to check whether a reaction was POSITIVE or NEGATIVE on agarose gel T / F
- d. There is no need to use positive or negative controls T / F

e. It is a highly sensitive detection assay

T / F

13. Name three items of Personal Protective Equipment that should be used whilst working with a potentially infected dog brain diagnostic submission or with live Rabies Virus in the laboratory.

14. Give two examples of Diagnostic tests that can be used to detect Influenza virus antigen in swap & tissue samples

15. Why is it important to include negative and positive controls each time you perform a diagnostic test

16. Which tests would you use to detect Influenza H7N9

- a. Type A PCR
- b. H7 PCR
- c. N9 PCR
- d. All of the above

17. Which test do you use to confirm H7N9

- a. H7 PCR
- b. N9 PCR
- c. HI Test
- d. Sequencing
- e. Virus Isolation

18. Which test is best to use detect ASF virus

- a. Real-time PCR
- b. Virus Isolation
- c. Serology
- d. All of the above.

19. What test can we use to detect CSF virus

- a. Serology
- b. Real-time PCR
- c. Virus Isolation
- d. Virus Isolation and Identification by antibody or PCR
- e. B & D
- f. All of the above.

20. What test can be used to detect PRRS

- a. Serology
- b. Real-time PCR
- c. Virus Isolation and Identification by antibody or PCR
- d. B & C
- e. All of the above.

ANNEX 9: Workshop Evaluation Form



POST EVENT EVALUATION FOR IDENTIFY-SUPPORTED ACTIVITIES



For participants - please provide comments where indicated and circle the number that best reflects your opinion:

A. Content and Quality	
1. Title of Event (<i>workshop/training/conference</i>): Regional Laboratory Network Training on Diagnosis of Swine Diseases	Date 27-31 August 2012
2. Content was relevant , up-to-date and applicable	<i>poor/not useful ...1 to 4...good/useful</i> 1 2 3 4
3. What new practical skills did you learn from the workshop/training?	
4. Balance between theory and practice? <i>Check here if not applicable</i> <input type="checkbox"/>	<i>Too much theory ...1 to 5...too much practice</i> 1 2 3 4 5
5. Time allocated to activities	<i>Not enough ...1 to 4...Sufficient</i> 1 2 3 4
6. To what aspects of your current role in your organization, and to what extent, will this workshop/training/conference contribute to improving?	<i>Not at all ...1 to 4...Completely</i> 1 2 3 4
7. To what extent would you say the training/workshop/conference met defined objectives?	<i>Not at all ...1 to 4...Completely</i> 1 2 3 4
8. To what extent would you say the training/workshop/conference met your expectations?	<i>Small extent ...1 to 4...Great extent</i> 1 2 3 4
9. What are your greatest needs in additional training for your laboratory?	
10. Of what significance was the workshop to improving regional networks (if applicable)	

B. Logistics and Organization (where applicable)		
10. Organization (presentation, materials, assistance e.t.c.)		<i>poor/not useful ...1 to 4...good/useful</i> 1 2 3 4
11. Invitation	<i>Check here if not applicable</i> <input type="checkbox"/>	<i>poor...1 to 4...good</i> 1 2 3 4
12. Flight arrangement	<i>Check here if not applicable</i> <input type="checkbox"/>	<i>poor ...1 to 4...good</i> 1 2 3 4
13. Airport to hotel transportation	<i>Check here if not applicable</i> <input type="checkbox"/>	<i>poor...1 to 4...good</i> 1 2 3 4
14. Accommodation	<i>Check here if not applicable</i> <input type="checkbox"/>	<i>poor ...1 to 4...good</i> 1 2 3 4
15. Venue / Room Facility	<i>Check here if not applicable</i> <input type="checkbox"/>	<i>poor/not useful ...1 to 4...good/useful</i> 1 2 3 4
16. Food and drink	<i>Check here if not applicable</i> <input type="checkbox"/>	<i>poor/not useful ...1 to 4...good/useful</i> 1 2 3 4
17. Supporting documentation and/or course materials	<i>Check here if not applicable</i> <input type="checkbox"/>	<i>poor/not useful ...1 to 4...good/useful</i> 1 2 3 4
C. Overall assessment		
18. General comments and your overall rating of the workshop/training/conference		<i>poor/not useful ...1 to 4...good/useful</i> 1 2 3 4
.....		
19. What would you have done differently?		
.....		

Participant information (OPTIONAL unless indicated)

Name/Title:	
Country:	
Current job /Organization:	
Time spent in current position:	
Contact phone/email:	
Sponsor(s):	