Report from the Regional Laboratory Network Training Workshop and Activities on the Diagnosis of Newcastle Disease (ND) and Rabies

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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
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 Livestock Development (DLD) Thailand to organize regional laboratory training workshop at the National Institute Animal Health (NIAH), Bangkok, Thailand. The laboratory-based training workshop was organized under the FAO Regional Laboratory Networking Strategy and was specifically designed to strengthen regional laboratory diagnostic and investigation capacities. The training workshop on the priority animal diseases Newcastle Disease (ND) and Rabies focused on the problems identified in the 2012 Proficiency Testing (PT) program, country backstopping missions and feedback from the TAG and Laboratory Directors meetings.

Specifically 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. Of particular significance, the workshop encompassed techniques and procedures that can be applied generically to the characterization of a wide range of viral agents.

The training covered the requirements for Rabies diagnosis from sampling the dog and the correct parts of the brain, introducing sampling using a straw versus the removal of the whole brain, to the testing for Rabies by Fluorescent Antibody Test (FAT), PCR, Rapid Test and the Immuno-peroxidase Test (IPX) which allows diagnosis using a normal light microscope. The training for Rabies serology looked at the use of the recommended cell culture immuno-techniques, the Rabies Rapid Fluorescent Foci Inhibition Test (RFFIT) and the Rabies Fluorescent Antibody Virus Neutralization (FAVN) test, and the Rabies antibody ELISA.

The ND training covered sample collection and the use of PCR, Virus Isolation (VI), Sequencing\Pathotyping and HA\HI for ND diagnosis, highlighting the need to understand the purpose of the test being used for detection of ND virus/antigen or antibody from field virus versus vaccine virus. The priority for ND is detection of field isolates and PCR Sequencing\Pathotyping is the best tests for detection and identification.

The workshop allowed all participants to gain hands on experience with known and unknown samples and controls (positive & negative) and allow them to gain experience in interpretation and trouble-shooting, especially with immuno-techniques and in setting up PCR tests. For Rabies diagnosis by immune-techniques (FAT & IPX) participants were given a number of opportunities to read unknown samples and give a result as a group and also individually, this allowed the facilitators to see if participants were able interpret the Rabies immuno - tests. 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 test results with a recommendation to the field veterinarians is an area where all laboratories need assistance and training. The training covered all the required diagnostic tests including the QA and Biosafety & Biosecurity requirements important for accurate test results and staff safety.

NIAH has also used the workshop to further establish Rabies Diagnosis at NIAH and have established the FAVN test for Rabies serology through the collaboration with AAHL in running the workshops in 2012 & 2013. NIAH 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.

In addition, training activities represented a unique training and networking opportunity for regional laboratory personnel. On conclusion, it was clearly apparent to the facilitators that the

participants had successfully established contacts with the host laboratory and other regional laboratories, strengthening the links between laboratories in the diagnostic laboratory network in the region.

The Laboratory Training Workshop was well received by all participants and formal feedback confirmed that the laboratory activities were highly successful. Participants felt the regional workshops not only gave them valuable training but also allowed them to discuss technical difficulties they experienced in their laboratories and discuss these with the other regional laboratories and AAHL. Participants see the laboratory network supported by AAHL as a important mechanism for them to solve technical problems. 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.

Also staff in a number of laboratories indicated that Rabies diagnosis is not well supported in the laboratory in regards to resources for testing, equipment, equipment maintenance/calibration and staff biosafety, e.g. some staff were not vaccinated and some laboratories do not have a BSC II cabinet for Rabies testing.

The information from the workshop was supplied on a USB to all participants.

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 Rabies and ND,
- Sharing update information on disease situation in the region and update diagnostic techniques
- Discussion on regional Rabies and ND 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, Malaysia, Myanmar, Thailand and Viet Nam took part in the training. Philippines was unable to attend. Participant list in Appendix 2.

5. Expected Outcomes

- Training
  - Animal health laboratories are capable of consistently and accurately diagnose Rabies and ND.
- 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 28th September 2012

- 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 information from the training workshops on Rabies and ND from the countries highlighted the continued need for harmonisation of diagnostic tests in the region and the need for ongoing PT and IQC to ensure that the results from tests are correct and consistant. The countries use different virus/antigen/genome detection tests/protocols, serological tests/protocols, reagents and QA/IQC controls which make it difficult to compare the results obtained in each laboratory. There is a ongoing need for regional PT and a need for regional and national QA/IQC reference controls (regional reference controls: low positive and negative IQC controls) for each test run to allow comparison of 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 to another test used in the region if they do not get the expected result for the Regional Reference control or to the regional test SOP if their test is found to be not performing. National Reference controls can be standardised against regional rerference controls and used to confirm national network laboratories are getting the same results.

The budget for reagents and to prepare and test IQC controls 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 in interpetation and trableshooting test results and gave participants the guidelines for Rabies and ND, diagnosis. The workshop discussions provided the current situation in the region for Rabies and ND, future directions and gaps. The workshop presented a diagnostic test alogrithm for each disease, for disease investeigation and surveillance (see Appendix 6 & 10) and a reccommended test SOP (provided as attachments to this report). Regional SOPs were based on AAHL SOPS, which are validated and based on OIE reccommended methods and validated tests from publications and reference laboratories.

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. NIAH from Thailand and RAHO – 6 from Vietnam, have accreditation to ISO17025 and others have no quality system in place Lao PDR and Cambodia. A QA system gives a laboratory a structure to implement test QA (especially use of IQC: test positive and negative controls), and ensure the laboratory has the systems (written in SOPS) in place to to ensure tests are giving accurate results 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 there committment to establishing a laboratory QA system and a safety culture for Biosafety and O.H & S.

AAHL staff acted as trainers and mentors and very successfully engaged with all the course participants, individuals who are likely to become their countries leaders in the field of animal health. NIAH 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. NIAH has also used the workshop to further establish Rabies Diagnosis

at NIAH and have established the FAVN test for Rabies serology through the collaboration with AAHL in running the workshops in 2012 & 2013.

A suggested activity from the workshop was for the laboratory network to work together to develop a regional realtime PCR for Rabies and NDV. The National laboratories would send Rabies and NDV samples/isolates to AAHL who will sequence the isolates and develop a realtime PCR which will then be evaluated by the regional laboratory network.

6.1 Rabies and NDV Training Workshop Bangkok, National Institute Animal Health (NIAH), Bangkok, Thailand.

The training for Rabies diagnosis covered the requirements for testing by FAT, PCR, rapid test and the immuno-peroxidase test (IPX) which allows diagnosis using a normal light microscope. The training Rabies serology looked at the comparison of the commercially available Bio- Rad Platelia Rabies ELISA kit with the Rabies Fluorescent Antibody Virus Neutralization (FAVN) test.

Currently Rabies Diagnostic testing which is recommended by the OIE involves the use of fluorescent conjugates which are commercially available at very high cost. The use of these commercially available detecting antibodies for serology and FAT also require expensive UV microscopes which are beyond the means of many Regional Laboratories in Asia to purchase and maintain. For this reason AAHL scientists have been working at producing lower cost Rabies virus detecting antibodies which can be used for this purpose without the need for expensive commercial antibodies and/or UV fluorescent microscopes.

The workshop allowed all participants to gain hands on experience with known and unknown samples and controls (positive & negative) and allow them to gain experience in interpretation and trouble-shooting, especially with immuno-techniques and in setting up PCR tests.

For Rabies diagnosis by immune-techniques (FAT & IPX) was a focus of the workshop with participants given a number of opportunities to read unknown samples and give a result as a group and also individually, this allowed the facilitators to see if participants were able interpret the Rabies immuno - tests. 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 test results using both laboratory and field information was a important in making a final diagnosis and report to the field (client : field veterinarian or farmer), with a recommendation to the field veterinarians is an area where all laboratories need assistance and training. The training covered all the required diagnostic tests including the QA and Biosafety & Biosecurity requirements important for accurate test results and staff safety.

Diagnosis of Rabies and ND using PCR, as part of the training course was conducted during the two-week period, with the aid of NIAH staff. For Rabies diagnosis, 4 PCR tests were introduced during the training. The first one is a conventional nested PCR detecting all members of lyssavirus genus, including Rabies virus. This test has been recommended as molecular diagnostic test for diagnosis of Rabies in the regional animal health laboratories. Three real-time TaqMan PCR assays for Rabies virus were also introduced and evaluated at the training. These assays are specific for Rabies virus. In comparison to the generic conventional nested PCR, these assays have advantages including high sensitivity and specificity, rapid turn-around time and very low risk of cross contamination during the testing procedure. However, based on our previous validation, these assay failed to detect certain rabies virus strains. Therefore, the real-time PCR assays are considered as complementary tests in addition to the conventional nested PCR assay for molecular

detection of rabies. The participants divided into 8 groups of two in each based on country origin, conducted both conventional nested PCR and all three real-time PCR assays on a panel of 10 samples, including 8 known positive and two known negative samples. The results demonstrated that Real-time PCR assay 1 was the most sensitive assay for detection of Rabies viruses tested, which was recommended as a complementary test to the conventional nested PCR for molecular diagnosis of Rabies in the regional laboratory network.

For ND diagnosis, two real-time TaqMan PCR assays, M gene assay and F gene assay, were conducted at the training. Both assays mainly detect Class II ND viruses (most virulent and avirulent strains, except some avirulent viruses from wild bird). The participants performed both PCR assays on a panel of 8 samples including 6 positive and two negative samples. The results demonstrated M gene PCR assay was more sensitive than F gene assay, which was in agreement with previous validation. The M gene PCR was recommended as primary test for molecular diagnosis of ND in regional laboratory network.

The participants learnt both principles and practice of molecular diagnosis of Rabies and ND through the training course. The participants performed all practical testing on designated sample panels following guidelines of trainers. Most of their testing results are close to that expected. There were some cross contamination issues during the testing procedure, partially due to the limited training facility provided, and the personal knowledge and skills. These need to be improved in the future practice in their own laboratories.

Objectives

1. This training program covered a range of laboratory tests available for the diagnosis of Rabies Virus infection under an ISO17025 framework. One of the main objectives was to develop and deliver low cost alternative laboratory tests for the antigen detection of Rabies Virus e.g. IPX Rabies test and Rabies Realtime PCR .
2. Examine the current tests used to detect NDV and recommend a SOP for detection of ND by Realtime PCR.

Pre-Training Workshop Test Development for Rabies

Part of the brief for this Rabies workshop was to develop and deliver new diagnostic tools for the region. Staff at AAHL have cloned the genetic code for the Rabies nuclear protein into an E.coli expression vector. Expressed protein was used to produce hyper immune serum in rabbits. This serum has been tested and validated by Immuno-histochemistry (IHC) on known Rabies infected brain tissues. The results so far have shown this serum to be both specific and sensitive for Rabies virus. The Histology unit at AHHL uses this serum for the Diagnosis of Rabies in fixed and thin section histology under our QA system ISO17025
Expanding on this we have developed tests which use Peroxidase as the detecting system. This eliminates the need for expensive Fluorescent upright and inverted microscopes in the diagnosis of Rabies. An alternative virus neutralization technique, Peroxidase Antibody Virus Neutralization (PAVN), and a antigen detection test, immuno-peroxidase (IPX) test, were presented at this course, as well as the OIE recommended FAT and FAVN tests. .

During the past year we have further developed the IPX test and have increased its sensitivity by changing the fixation method, adding a hydrogen peroxide blocking and counter staining step which we delivered during this course This test can be read on an inverted light

microscope. Similarly for Antigen detection in Rabies brain smears, the FAT which is the gold standard test for Rabies diagnosis can be replaced by the IPX test.

To expand on last years Rabies PCR activities we investigated the Real Time PCR assays as described by Nadin-Davis paper. This paper describes three separate TaqMan assays all having primers and probes that are homologous to conserved regions of the lyssavirus N gene with Thailand Rabies strains to ascertain the suitability of these assays for the region.

Workshop Activities:

- Workbook for Rabies Diagnosis was delivered with activities, work schedule as well as some trouble shooting guidelines for each of the modules delivered. Explanations for reasons the new tests were developed and the scope of these tests was discussed with participants.

- Provide training in the detection of Rabies Antibodies in serum
  - Rabies Bio Rad Platelia ELISA kit
  - Result Analysis using spreadsheets and reporting International Units/ml

- Provide training in the detection of Rabies antigen in brain tissue
  - Rabies FAT (OIE recommended AAHL validated test)
  - Rabies IPX (development stage- not validated)
  - RT-PCR Conventional (OIE recommended AAHL validated test)
  - Real-time Rabies PCR

- General overview of the requirements of ISO17025
  - Use of positive and negative controls
  - Test Coversheets, reagent monitoring and recording
  - Reporting guidelines for Rabies diagnosis

- Biosafety
  - Vaccination guidelines by the WHO for staff handling Rabies
  - Rabies Laboratory requirements for safe work practice
  - Handling Zoonotic Agents, storage & waste disposal

- Proficiency Panels composition

A lot of background preparation by AAHL staff went into the composition of the four rabies proficiency panels prepared for the region. The Rabies panels were new to the regional PT panels and the production of a range of samples to test sensitivity and specificity and to ensure that staff were competent was difficult. Ensuring stability of the Rabies FAT & IPX panels in particular was important to ensure samples were still working when received by the laboratories.

The serum panel consisted of dog sera from vaccinated and unvaccinated dogs where the Rabies titre in IU/ml had been determined at AAHL under a QA system using the FAVN test and as such a precise known titre of each serum is known. In this way all serological tests conducted from ELISA kits, RFFIT, FAVN could be compared against each other

The FAT and IPX dog brain smear panels were prepared at AAHL prior to the course. Andrea Certoma and NIAH staff supplemented the panles with positive and negative dog brain smears from Region specific brains held at NIAH. This also provided and excellent opportunity for NIAH staff to be involved in the production of FAT and IPX brain smear positive and negative controls for use as test IQC controls or as PT samples. The use of rabies positive brains from Thailand also ensured these panels were fit for purpose and represented rabies strains circulating in the region.

The PCR panel consisted of 10% dog brain homogenate of Gamma Irradiated South African Rabies infected dog brains, region specific Rabies dog brain and Australian Bat Lyssavirus (Pteropid) as the differential strain.

- Delivery of Proficiency panels
  - Rabies Serum panel for ELISA or Virus Neutralization Test
  - FAT Panel of fixed brain smears with reagents to conduct the testing
  - IPX Panel of fixed brain smears with reagents to conduct the testing
  - PCR panel with primers
  - Report forms with testing instructions and requirements to be filled out and returned by the 27th September 2013

- Trouble shooting strategies were covered for the different tests

- Delivery of amended SOP method for IPX antigen detection for Rabies virus

- The ND training covered the use of a PCR, for ND diagnosis, highlighting the need to understand the purpose of the test being used for detection of ND virus/antigen or antibody from field virus/antibody versus vaccine virus/antibody.

  - NDV antigen detection using PCR covered the different PCR test that can be used, e.g. PCR to detect all NDV isolates versus a test to detect only field virus.
  - For ND diagnosis, two real-time TaqMan PCR assays, M gene assay and F gene assay, were conducted at the training. Both assays mainly detect Class II ND viruses (most virulent and avirulent strains, except some avirulent viruses from wild bird).

Regional protocols for diagnosis and characterization of Rabies and ND.

Rabies Diagnostic Test Algorithm:

The Diagnostic test algorithm for Rabies (Annex 9) outlines the diagnostic process for Rabies surveillance and investigation. The recommended samples for a Rabies investigation are brain, salivary glands and saliva; 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 FAT is the recommended test for detection of Rabies antigen using specific rabies antibody that has been validated and optimized to give maximum sensitivity and specificity.

A conventional RT-PCR test for detection of Rabies genome using primers that have been validated and optimized to give maximum sensitivity and specificity can be used as a confirmation test.

The recommended regional protocol for Rabies, the FAT is attached for reference; it is based on the AAHL/OIE 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 FAT. PCR is a test that can be used for confirmation of Rabies. The use of PCR, rapid test or IPX needs to be validated for use, e.g. in province laboratories, where the FAT cannot be put in place. A country needs to have the Rabies FAT in place at the national reference laboratory as confirmation to use of other tests.

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. Rabies 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 Rabies virus isolates and where the virus came from.

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

The training workshop provided training in FAT and immuno-peroxidase staining for Rabies for identification of Rabies... A module on the Bio Rad Platelia ELISA's suitability for surveillance of dog populations was delivered. The conventional Rabies PCR test was reviewed and the Real Time Taqman assays developed by (Nadin-Davis et al., 2009). The use of immuno-peroxidase techniques and cheaper antibodies were reviewed in this workshop to examine if these techniques can replace current immuno-florescent techniques.

NDV Diagnostic Test Algorithm:

The Diagnostic test algorithm for ND (Annex 10) outlines the diagnostic process for ND surveillance and investigation. The recommended samples for a ND investigation are tracheal and cloacal 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 ND diagnosis, two real-time TaqMan PCR assays, M gene assay and F gene assay, were conducted at the training. Both assays mainly detect Class II ND viruses (most virulent and avirulent strains, except some avirulent viruses from wild bird). The M gene Real-time RT-PCR is the recommended test for detection of ND antigen/genome using primers/probes that have been validated and optimized to give maximum sensitivity and specificity. The F gene assay is used as a second test to confirm suspect ND samples.

The recommended regional protocol for ND TaqMan Assay is attached (Attachment 2) 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 Real-time RT-PCR and

the capability to produce cDNA. NDV VI and identification protocols were made available to the participants of the workshop and are used by some laboratories and the primary diagnostic test. VI and HA/HI using a specific NDV antibody can take up to 14 days to identify the disease agent and requires the correct reference antibody. ND PCR also requires the correct test but offers same day identification.

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 NDV virus isolates and where the virus came from.

Serology for ND uses the HI test to detect NDV antibody. Laboratories have to ensure they are using the correct antigen for the HI test for post-vaccination surveillance and NDV antibody detection.

7. Training and workshop evaluation

A set of test questions and an evaluation form (Annex 11 & 13) was given to each participant after the training and workshop. The results from the test questions for the Rabies Workshop are given in Annex 12. The results from the evaluation forms are are attached to thid report for the training workshop.

All the participants agreed that the training workshops were useful and were satisfied with the workshop and training. The general feedback included: the participants were very happy with the was more hands on training in this workshop trainers were excellent and good cooperation of NIAH staff and enhanced relationships among countries.

There were still some 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.

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.

There was great interest in the non-fluorescent detection systems which were developed for the Region participants were keen to try less expensive commercial conjugates for the Rabies FAT and the IPX that did not rely on a Fluorescent microscope.

8. Conclusions and recommendation

The workshop participants agree that a harmonised approach to disease diagnosis and the implementation of QA in the laboratories was the best approach. Regional guidelines and harmonized protocols for the diagnosis and molecular characterisation of agents in animals supplied to and used by member countries was the best approach to 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 and in problem-solving. The back-stopping missions help provide ongoing in-country support, problem-solving and capacity building to the whole laboratory .

The laboratory participants and experts discussed testing algorithm for Rabies, ND, ASF, PRRS and CSF for surveillance and investigation and these have been finalised and included in this report (Annex 6, 7, 8, 9, & 10), outlining that for most diseases the primary diagnostic test for disease diagnosis is a specific Real-time PCR, e.g. AI, ND, 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 or a range of positive samples should be referred to a reference laboratory for virus isolation and further characterisation. For Rabies the FAT is the primary test for detection of Rabies but PCR is very useful for confirmation.

The hands on approach to training along with updates on the disease situation and discussions of test procedures was a successful tried format and gave the best results in ensuring uptake from the participants. Having the participants repeatedly interpret results from unknown samples gave both the participants confidence they were performing the tests and the facilitators confident the participants were able to get the correct results. Hand s on training also made it easier to troubleshoot problems that maybe going worry with the tests.

The development of a non-fluorescent test (IPX) and or a Real-time PCR for Rabies diagnosis along with cheaper reagents will be very useful for the region.

It was agreed that for serology an ELISA test for Rabies is the best test for surveillance for antibody and for post-vaccination surveillance. The ELISA test used needs to be a validated test (see OIE for the recommended ELISA test: e.g. Bio-Rad Platelia ELISA). The VNT test (RFFIT and FAVN) for antibody is seen as the gold standard and needs to be available in reference and key laboratories for confirmation of ELISA results.
For ND the HI test is the best available test for detection of NDV antibody and laboratories have this test in routine use but did not know how well their test performed. The results from the IDENTIFY PT panel for ND serology have shown most laboratories had a working NDV HI test in place in there laboratory with some improvement in sensitivity needed. The lack of reference controls (IQC) used in the region for NDV meant the laboratory had no way of knowing there NDV HI test was working, the NDV HI PT gave the laboratories this information. An ongoing regional reference control and sharing of information will help harmonise results in the region. For the short term using the PT Reference positive control can help compare laboratory results.

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 continued focus on ensuring laboratory staff at each laboratory had a good understanding of what is required for 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. Laboratories needed to ensure that they planned for testing BSCII cabinets (currently done for FAO). The area of most concern in the laboratory is the post-mortem rooms, with most rooms having poor biosafety/biosecurity and lacking in budget to supply PPE and disinfectant to these areas. There is a lack of focus on these areas by the laboratory with most needing to write working SOP to improve practices and to allocate a budget to improve PM rooms, especially where zoonotic and possible zoonotic agents are worked with.

For NDV antigen detect the current Realtime PCRs are not specific for NDV field isolates and means that to determine if a sample from the field is ND from vaccine or virlent ND, the laboratory needs to do sequencing. Most laboratories do not have this capacity. There is a need for a Realtime PCR or a PCR that detects only field isolates.

A suggested activity from the workshop was for the laboratory network to work together to develop a regional realtime PCR for Rabies and NDV. The National laboratories would send Rabies and NDV samples/isolates to AAHL who will sequence the isolates and develop a realtime PCR which will then be evaluated by the regional laboratory network.

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 and have been attached in this report..

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. 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 workshop 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. Through the Director forum the uptake of training after workshops has improved with better support to staff who attended the workshops to use and share the information on return to the laboratory. 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: Rabies & NDV Training Schedule

WEEK ONE SCHEDULE					
TIME	**Monday 26th Aug**	**Tuesday 27th Aug**	**Wednesday 28th Aug**	**Thursday 29th Aug**	**Friday 30th Aug**
8.30 – 12.30	**Welcome & Introduction** NIAH presentation **Outline of Workshop** • Aims • Agenda • Biosafety • QA & ISO17025 **Overview of NIAH** **Lectures** • NDV Disease& Diagnostics • Rabies Disease & Diagnostics	**Newcastle Disease Virus(NDV) (Blue Group)** **NDV Taqman PCR** • Discussion NDV PCR fit for purpose • PCR optimization **Rabies: (Green Group)** **Buffers & Reagents** • Water quality • pH testing • Preparation & Coating AAS slides **Rabies FAT** • Conjugate comparison	**Rabies** **Rabies PCR Day 1: (Blue Group)** • RNA extraction • 1^0 PCR reaction **Rabies Indirect FAT: (Green Group)** • Lecture • Practical	**Rabies** **Rabies RIAD/IPX: (Green Group)** • Lecture • practical **Rabies PCR Day 2 (Blue Group)** • 2^o Reaction • Gel electrophoresis	**Rabies** • Reading FAT and RIAD slides **Review Week 1 results** • NDV Taqman • Rabies Conventional PCR • Reading FAT and RIAD slides **Troubleshooting** **Quality Assurance & IQC** **Biosafety & Biosecurity**
1.30 – 4.30	**Biosafety & Biosecurity** • NIAH procedures • General Requirements • Working in a BSCII Cabinet **Rabies sample collection and preparation** • Pathology/Post Mortem • Rabies brain dissection and smear preparation • Prepare 10% homogenate • Prepare FAT/IPX Slides	**Rabies: (Blue Group)** **Buffers & Reagents** • Water quality • pH testing • Preparation & Coating AAS slides **Rabies FAT** • Conjugate comparison **Newcastle DiseaseVirus (NDV) (Green Group)** **NDV Taqman PCR** • Discussion NDV PCR fit for purpose • PCR optimization	**Rabies** **Rabies PCR Day 1: (Green Group)** • RNA extraction • 1^0 PCR reaction **Rabies Indirect FAT: (Blue Group)** • Lecture • Practical	**Rabies** **Rabies PCR Day 2: (Green Group)** • 2^o Reaction • Gel electrophoresis **Rabies RIAD/IPX: (Blue Group)** • Lecture • practical	**NDV Serology** • Review of PT results 2012 **PCR** • Test Optimization • Review PT results for NDV,AI,CSF,ASF, PRRS & Rabies **Establishing a new diagnostic test** • Equivalency and Validation

WEEK TWO SCHEDULE					
TIME	**Monday 2nd Sep**	**Tuesday 3rd Sep**	**Wednesday 4th Sep**	**Thursday 5th Sep**	**Friday 6th Sep**
8.30 – 12.00	**Rabies** **Rabies FAT: (Green Group)** • Bio Rad-NIAH Method • Indirect FAT • Comparison of Direct & Indirect FAT **Rabies PCR: (Blue Group)** • Rabies Real-time PCR • Discussion on Rabies Real-time PCR tests	**Rabies** **Rabies ELISA: (Blue Group)** • Discuss Rabies Serology **Rabies RIAD/IPX: (Green Group)**	**Rabies** **Rabies ELISA: (Green Group)** • Discuss Rabies Serology **Rabies RIAD/IPX: (Blue Group)**	**Review Week 2 results** • Rabies Taqman PCR • Reading FAT and RIAD slides • Rabies ELISA results **Troubleshooting** **Quality Assurance & IQC**	**Proficiency Test** • Participants to read slides from unknown samples • Interpretation Laboratory Results **Workshop Review** • Post-workshop work plan • Purpose of tests • Constraints **Electronic copies of SOPs, methods & results** **Post-course Questionnaire**
01.00 – 16.30	**Rabies FAT: (Blue Group)** • Bio Rad-NIAH Method • Indirect FAT • Comparison of Direct & Indirect FAT **Rabies PCR: (Green Group)** • Rabies Real-time PCR • Discussion on Rabies Real-time PCR tests	**Rabies RIAD Continued (Green Group)** • Slide interpretation **Laboratory Workflow: (Blue Group)** • Tour of NIAH laboratory	**Rabies RIAD Continued: (Blue Group)** • Slide interpretation **Laboratory Workflow: (Green Group)** • Tour of NIAH laboratory	**Post Course Testing Requirements** • Rabies RIAD kit • Rabies FAT reagents **Rabies & NDV PCR reagents** **Proficiency Panels instructions** **Update H7N9**	**Proficiency Panel delivery** • Instruction on testing required and due date **Conclusion & Presentations** **Closing Ceremony**

ANNEX 2: Rabies and ND Workshop Participants List

Regional laboratory Network Workshop on Diagnosis of Rabies and Newcastle Disease

26 August-6 September 2013
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Regional laboratory Network Workshop on Diagnosis of Rabies and Newcastle Disease

26 August-6 September 2013
The National Institute of Animal Health, Bangkok, Thailand

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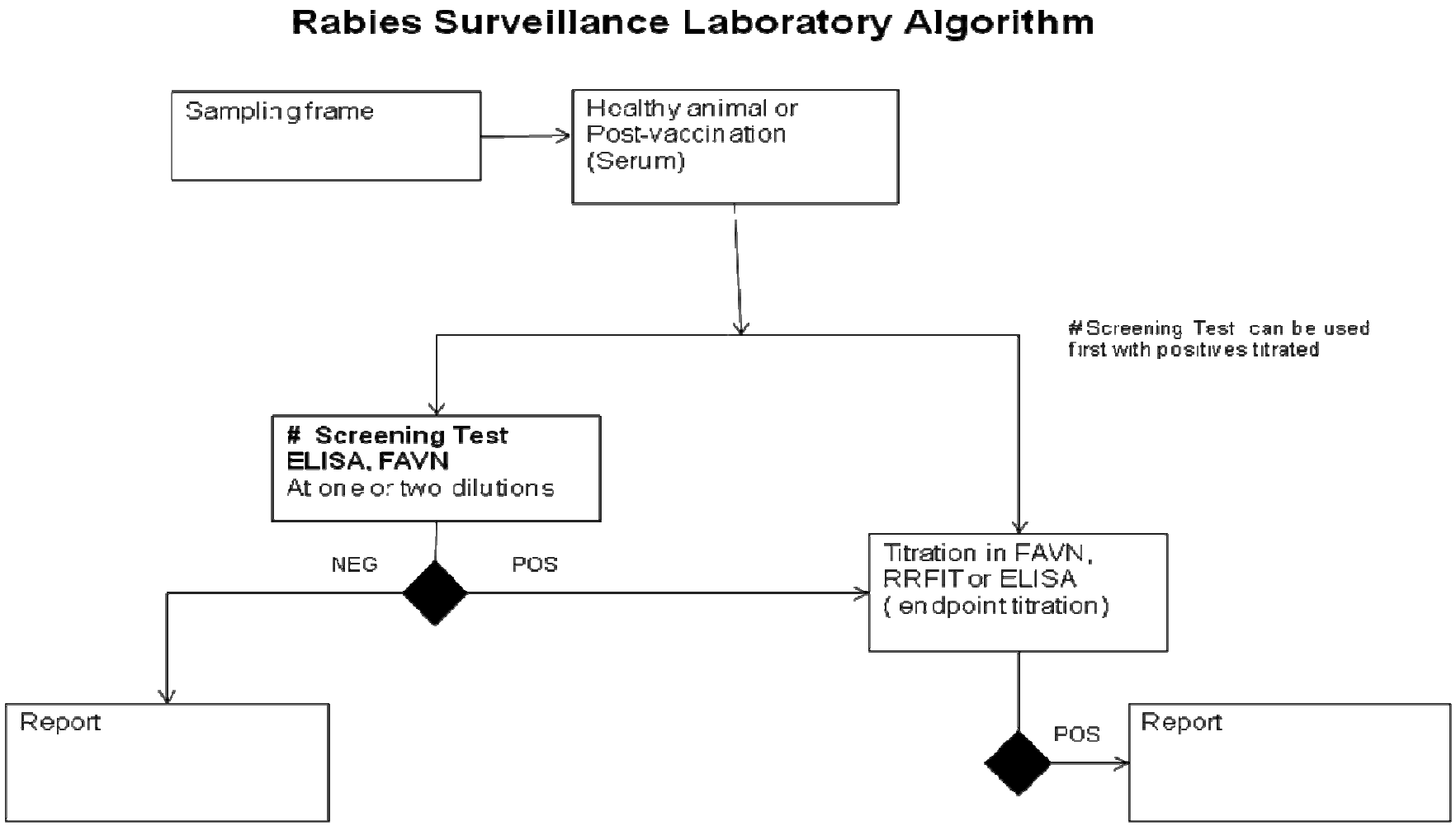
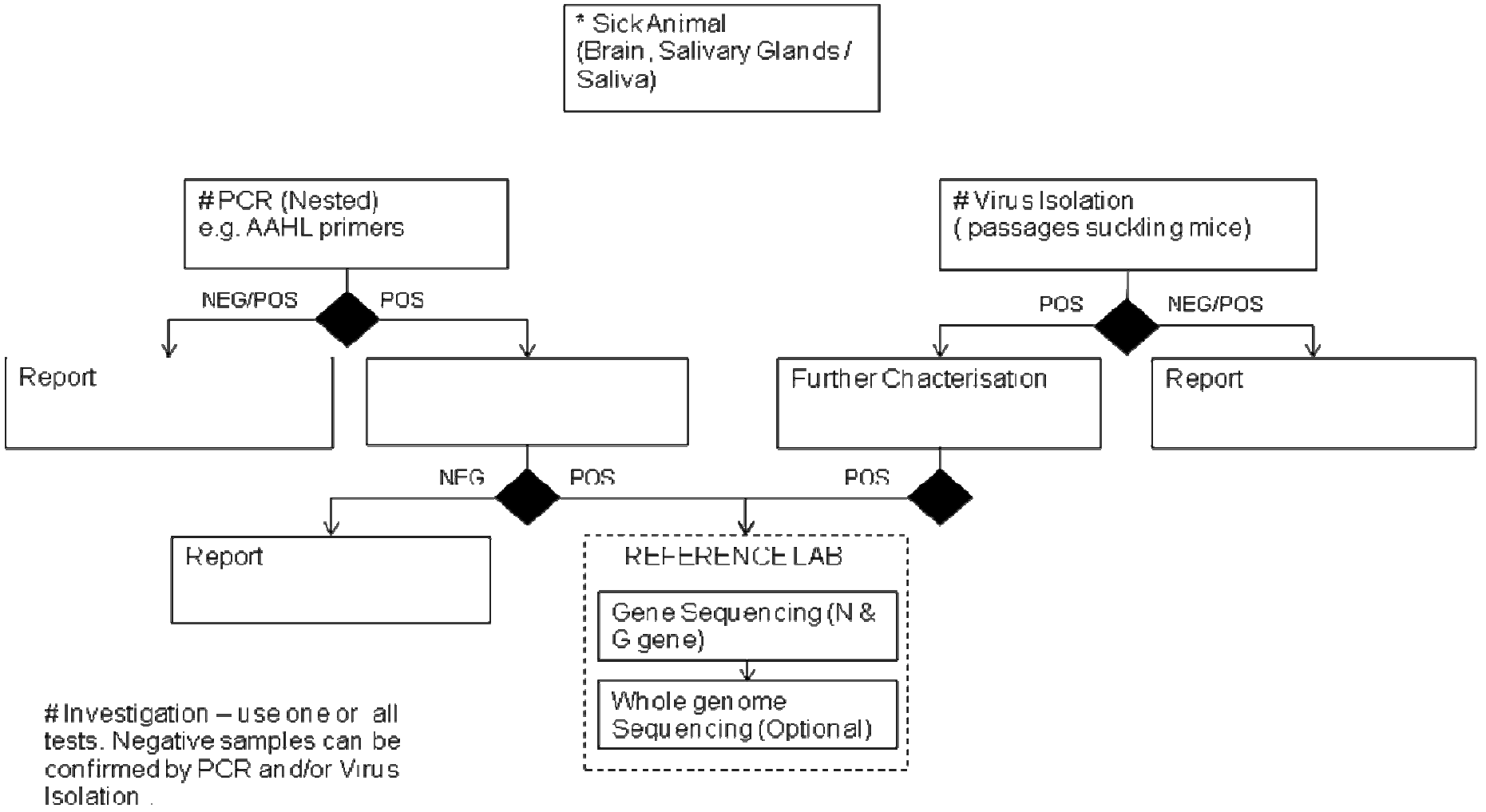
Annex 3: Rabies Diagnostic Test Algorithm for Surveillance & Investigation

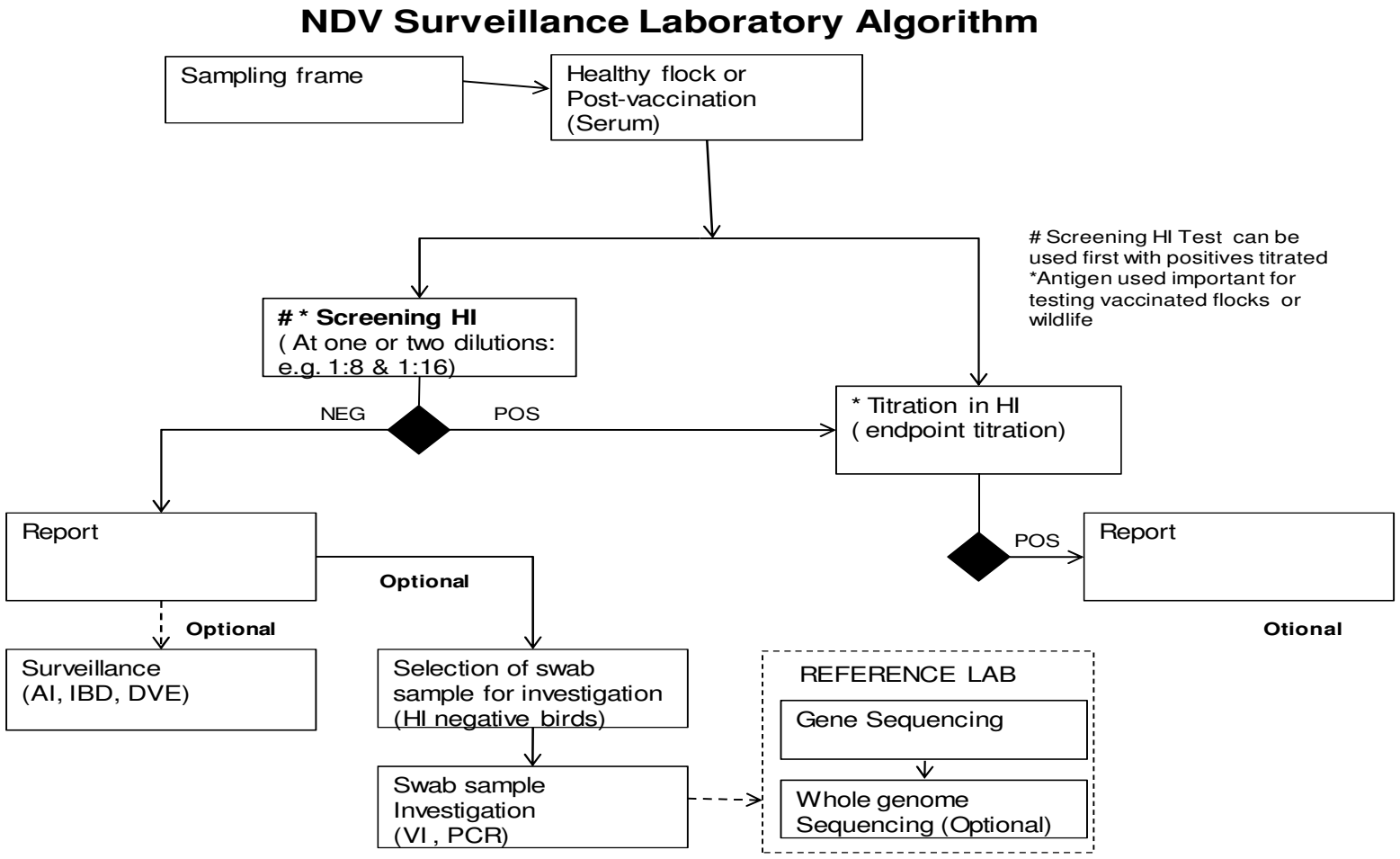


Rabies Investigation Laboratory Algorithm

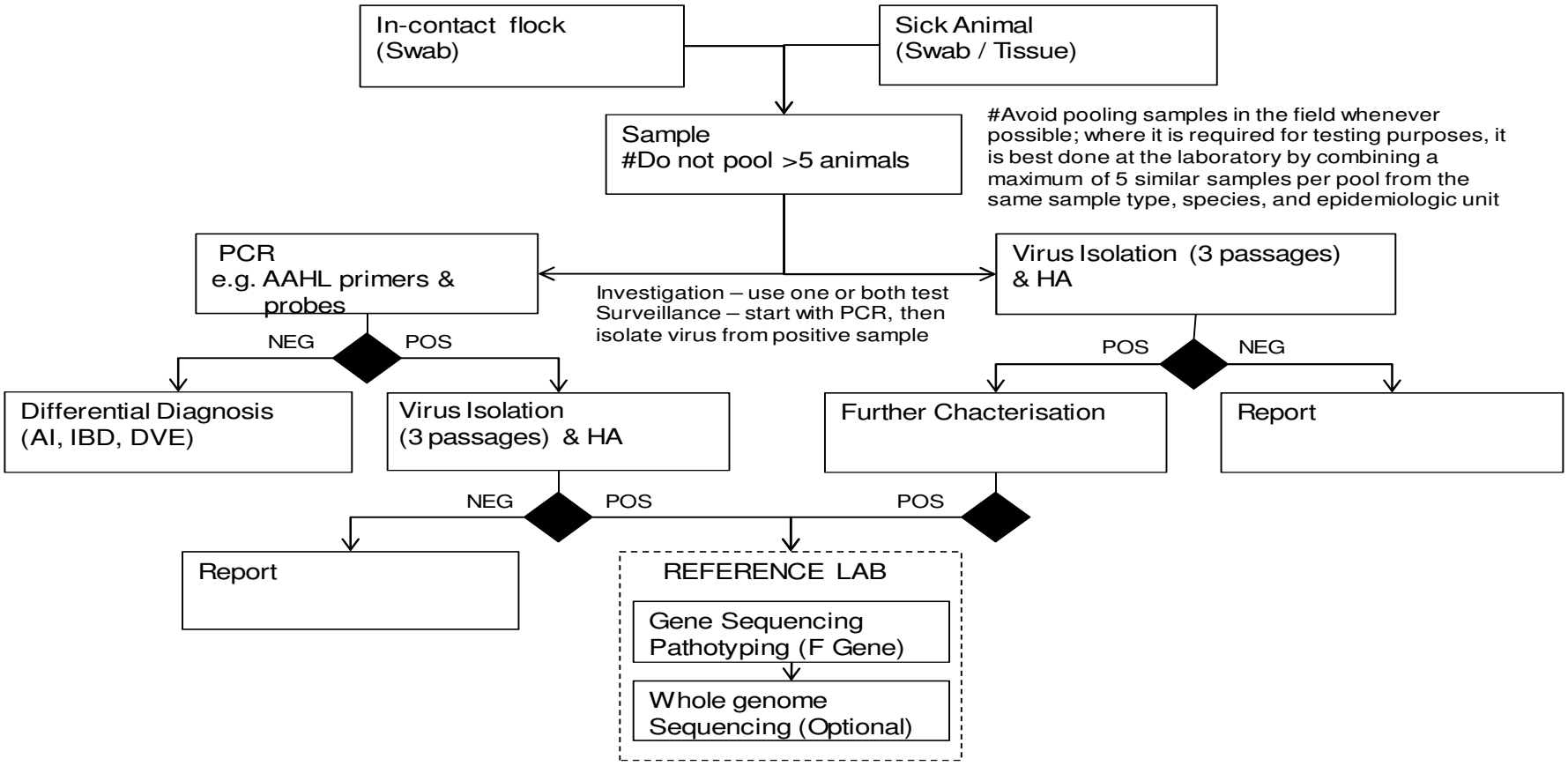


Investigation – use one or all tests. Negative samples can be confirmed by PCR and/or Virus Isolation .

Annex 4: ND Diagnostic Test Algorithm for Surveillance & Investigation



NDV Investigation Laboratory Algorithm



ANNEX 5

Questionnaire for Rabies & NDV Training Course DIC Bangkok Thailand 2013

1. Name three items of Personal Protective Equipment that should be used whilst working with a potentially infected dog brain diagnostic submission
    a. _____
    b. _____
    c. _____
2. List 5 types of equipment that should be calibrated in the laboratory, and how often should this be done?
    a. _____ calibration interval:_____
    b. _____ calibration interval:_____
    c. _____ calibration interval:_____
    d. _____ calibration interval:_____
    e. _____ calibration interval:_____
3. What is the difference between a laminar flow cabinet and a Class II biological safety cabinet?
4. You test the pH of the distilled water in your laboratory and it is found to be 5.9 what would you do?
5. What should the pH of distilled water be? pH_____
6. What temperature and time duration should you heat inactivate diagnostic serum samples before testing in the laboratory?
    - Temperature:_____
    - Time:_____
7. What control standards should be included in a PCR test?
8. What is important when establishing a Real Time PCR test in your laboratory?
    a. Reference controls
    b. Testing local field samples

c. Setting the "Cut off" value
d. Establishing the set point for the Threshold
e. Doing a titration for sensitivity
f. a & e
g. All of the above

9. 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

10. Which test is best to use to detect Newcastle Disease Virus (NDV)?
a. Real-time PCR
b. Virus Isolation
c. HI test
d. All of the above.

11. What is important in detecting NDV antibody for protection after vaccination?
a. Reference sera
b. Vaccine virus
c. Reference field virus
d. a & c
e. All of the above

12. What should you consider when deciding on an appropriate PCR test for NDV "Fit for Purpose"?
13. What conclusion can be drawn when a sample gives a positive Rabies PCR result but a negative Rabies FAT result?
14. Which test is the gold standard test used to use detect Rabies Virus in a sample?
    a. Real-time PCR
    b. FAT
    c. Virus Isolation
    d. Conventional PCR
    e. RFFIT
    f. All of the above.
15. What tests can you use to detect Rabies virus antibodies in a serum sample?
    a. Rabies Fluorescent Foci Inhibition Test(RFFIT)
    b. Fluorescent Antibody Virus Neutralization (FAVN)
    c. Haemaglutinating Inhibition Test (HI)
    d. ELISA
    e. a, b & d
    f. all of the above
16. What test would you use to confirm the presence of Rabies virus in sample?
    a. Real Time PCR
    b. Conventional PCR
    c. Sequencing
    d. ELISA
    e. FAT
    f. b & c
17. Why is it important to include negative and positive controls each time you perform a diagnostic test?

Participants Name: **Representing Laboratory:**

Date: **Result: /**

ANNEX 6: Results Pre/Post Rabies/ND Course Technical Questionnaire Results

Pre Course Results

Response		QUESTION/AVAILABLE MARKS																	
		Q1/3	Q2/10	Q3/2	Q4/1	Q5/1	Q6/2	Q7/4	Q8/1	Q9/5	Q10/1	Q11/1	Q12/3	Q13/1	Q14/1	Q15/1	Q16/1	Q17/1	TOTAL/38
A	Sintarato	2	4	0	0	1	0	3	1	0	1	0	0	0	0	0	1	0	10
B	Kansec	2	1	2	0	1	1	0	1	4	0	0	0	0	1	0	0	0	13
C	Limwibulpong	2	7	2	0	1	1	3	0	4	1	1	0	0	1	1	0	0	21
D	D	3	5	2	1	1	1	2	1	3	1	0	0	0	1	0	0	1	20
E	E	3	8	0	0	0	1	2	1	3	1	0	0	0	1	0	0	1	19
F	weai Levin (myanmar)	3	3	0	0	1	0	0	1	1	0	0	0	1	0	0	0	0	10
G	Vo Van Hung	3	8	2	0	1	2	3	1	4	1	1	1	1	1	1	1	0	28
H	H	3	3	0	0	0	0	0	1	4	0	0	0	0	0	0	0	0	11
I	Su Khin (myanmar)	3	5	0	1	1	0	0	1	0	0	0	0	0	0	0	1	0	12
J	J	3	3	2	0	1	2	2	1	0	0	0	1	1	0	0	0	1	15
K	Hao Vn	3	10	2	0	1	2	3	1	4	1	1	0	1	0	1	1	0	28
L	Agus	3	10	1	1	1	1	3	1	4	1	0	0	1	1	1	1	1	28
M	M	3	9	2	1	1	1	3	1	4	0	0	0	1	1	1	1	1	27
N	Tanrattanawong	3	4	1	0	1	2	3	1	0	1	1	0	0	0	1	0	0	15
O	Majina	3	6	0	1	1	2	0	1	5	0	1	0	0	1	0	1	0	22
P	Treevisai	3	9	0	1	1	2	0	0	5	1	0	0	0	1	0	0	0	23
Q	Chanafy	3	9	0	1	1	2	0	0	5	1	0	0	1	0	0	0	0	23
R	R	3	10	2	0	1	2	4	1	4	1	0	0	0	1	1	1	1	28
S	Dokphut	3	8	0	1	1	2	0	1	3	1	0	0	0	0	0	0	0	20
T	Norasura	3	6	2	0	1	0	1	0	0	1	1	0	0	1	1	0	0	16
U	Sothearos	3	5	1	1	1	1	2	1	3	1	0	0	0	1	0	0	1	19

Post Course Results

Response		QUESTION/AVAILABLE MARKS																	
		Q1/3	Q2/10	Q3/2	Q4/1	Q5/1	Q6/2	Q7/3	Q8/1	Q9/5	Q10/1	Q11/1	Q12/1	Q13/1	Q14/1	Q15/1	Q16/1	Q17/1	TOTAL/38
A	Sintarato	3	9	0	0	1	2	4	1	4	1	1	0	0	1	0	0	1	28
B	Kansec	2	7	2	1	1	2	4	1	5	1	0	0	1	1	0	0	1	29
C	Limwibulpong	3	7	1	1	1	2	4	1	5	0	1	0	1	1	0	1	1	30
D	D	3	9	2	1	1	2	3	1	5	1	1	2	1	1	1	1	1	36
E	E	3	8	2	1	1	2	3	1	5	0	0	1	1	1	0	0	1	30
F	weai Levin (myanmar)	2	5	1	1	1	2	2	0	5	0	0	0	0	1	0	0	1	21
G	Vo Van Hung	3	10	2	1	1	1	3	0	3	1	1	0	1	1	1	1	1	31
H	H	3	10	0.5	1	1	2	2	0	5	0	1	0	1	1	0	0	1	28.5
I	Su Khin (myanmar)	3	5	1	1	1	1	2	1	5	1	0	0	0	1	0	0	1	23
J	J	3	8	2	1	1	2	3	0	5	0	0	0	0	1	1	0	1	28
M	M	3	8	2	1	0	2	3	0	5	0	0	0	0	1	1	0	1	27
N	Tanrattanawong	3	10	2	1	1	1	2	1	4	1	1	2	1	1	1	1	1	34
O	Majina																		0
P	Treevisai	3	5	0	1	1	2	0	1	5	1	0	0	1	1	1	0	1	23
Q	Chanafy																		0
R	R	3	10	2	1	1	2	4	1	4	0	0	3	0.5	1	1	0	1	34.5
S	Do Thi Hoa	3	9	1	0	1	2	3	0	3	1	1	0	1	1	1	1	0	28
T	T	3	0	2	1	1	1	3	1	5	0	0	2	1	1	1	1	1	24
U	Phouvong	3	9	2	0	1	2	3	1	5	1	0	0	1	1	1	0	1	31
V	V	3	5	0.5	0	1	2	2	1	4	1	0	0	1	0	0	0	1	21.5
W	Sarinya	3	6	1	1	1	2	3	0	5	0	1	0	0	1	0	0	0	24
X	Tapakorn	3	6	0	1	1	2	0	1	5	1	0	0	1	1	1	0	1	24

Questionnaire: Average Results

Question #	Available marks	Pre-Course Average mark	Post-Course Average mark	% Improvement
1	3	2.857	2.9	
2	10	6.333	7.3	
3	2	1.000	1.3	
4	1	0.429	0.8	
5	1	0.905	0.95	
6	2	1.190	1.8	
7	3	0	2.65	
8	1	0.810	0.65	
9	5	2.857	4.6	
10	1	0.667	0.55	
11	1	0.286	0.4	
12	3	0.095	0.5	
13	2	0.333	0.675	
14	1	0.571	0.95	
15	1	0.381	0.55	
16	1	0.381	0.3	
17	1	0.333	0.9	
Total	38	19.429	25.25	

ANNEX 7: Rabies & NDV reagents suppleid to Participants

RIAD Kit Contents

RABIES IMMUNOPEROXIDASE ANTIGEN DETECTION
(RIAD/IPX) KIT

SUPPLIED IN THIS KIT

REAGENT	AMOUNT
1^0 Antibody anti- Rabies #663	50µL
2^0 Antibody Jackson conjugate- FITC	50µL
20X DAKO Tris pH7.6 concentrate	200mL
Skim milk powder	5g
Acetate buffer pH5.0	40mL
Scott's Tap water	15mL
Humidity Chamber	1
N,N Dimethylformamide (DMF)	2mL
AEC 4 vials	2mg/vial
Haemotoxylin	20mL
Aqueous Mounting medium	2ml
RIAD/IPX Proficiency panel	1
30% Hydrogen Peroxide H_2O_2	4mL

Fluorescent Antibody Test (FAT)

Reagents Supplied

REAGENT	AMOUNT
BioRad anti-Rabies conjugate	3ml
DAKO Fluorescent Mounting medium	2ml

Not Included (Laboratory to supply)
Light microscope
Coverslips
Personal Protective Equipment

PCR Reagents supplied

NDV Realtime PCR Primers & Probe

Rabies Realtime PCR Primers & Probe
Rabies Conventional PCR Primer

ANNEX 8: FAT RESULT READING 6.9.13

Expected

SLIDE #	FAT TEST DESCRIPTION	EXPECTED RESULT
1	Fujirebio conjugate	Positive
2	Fujirebio conjugate	Negative
3	Indirect FAT	Positive

Country	Participant	SLIDE 1	SLIDE 2	SLIDE 3
Thailand NIAH (1)	1	Positive	Negative	Positive
	2	Positive	Negative	Positive
Myanmar	1	Positive	Positive	Positive
	2	Positive	Negative	Positive
Cambodia	1	Positive	Negative	Positive
	2	Positive	Negative	unable to read
Viet Nam	1	Positive	Negative	Positive
	2	Positive	Negative	Positive
Indonesia	1	Positive	Negative	Positive
	2	Positive	Negative	Positive
Malaysia	1	Positive	Negative	Positive
	2	Positive	Negative	Positive
Thai Regional(1)	1	Positive	Negative	Positive
	2	Positive	Negative	Positive
Lao PDR	1	Positive	Negative	Positive
	2	Positive	Negative	Positive
Thai Regional(2)	1	Positive	Negative	Positive
	2	Positive	Negative	Positive
Thailand NIAH (2)	1	Positive	Negative	Positive
	2	Positive	Negative	Positive

ANNEX 9: RIAD RESULT READING 6.9.13

SLIDE	DESCRIPTION	Expected Result
A	PT4 Positive	Positive
B	Thai Positive	Positive
C	PT5 Positive	Positive
D	PT1 Negative	Negative
E	Thai Neg*	Negative
F	Negative control	Negative
G	Positive control	Positive
* This brain was PCR positive but FAT negative		

		SLIDE						
Country	**Participant**	**A**	**B**	**C**	**D**	**E**	**F**	**G**
Thai NIAH (1)	1	Positive	Positive	Positive	Negative	Negative	Negative	Positive
	2	Positive	Positive	Positive	Negative	Negative	Negative	Positive
Thai NIAH(2)	1	Positive	Positive	Positive	Negative	Negative	Negative	Positive
	2	Positive	Positive	Positive	Negative	Negative	Negative	Positive
Viet Nam	1	Positive	Positive	Positive	Negative	Negative	Negative	Positive
	2	Positive	Positive	Positive	Negative	Negative	Negative	Positive
Lao PDR	1	Positive	Positive	Positive	Negative	Negative	Negative	Positive
	2	Positive	Positive	Positive	Negative	Negative	Negative	Positive
Indonesia	1	Positive	Positive	Positive	Negative	Negative	Negative	Positive
	2	Positive	Positive	Positive	Negative	Negative	Negative	Positive
Cambodia	1	Positive	Positive	Positive	Negative	Negative	Negative	Positive
	2	Positive	Positive	Positive	Negative	Negative	Negative	Positive
Malaysia	1	Positive	Positive	Positive	Negative	Negative	Negative	Positive
	2	Positive	Positive	Positive	Negative	Negative	Negative	Positive
Myanmar	1	Positive	Positive	Positive	Negative	Negative	Negative	Positive
	2	Positive	Positive	Positive	Negative	Negative	Negative	Positive
Thai Regional	1	Positive	Positive	Positive	Negative	Negative	Negative	Positive
	2	Positive	Positive	Positive	Negative	Negative	Negative	Positive

ANNEX 10: Workshop Evaluation Form

POST EVENT EVALUATION FOR IDENTIFY-SUPPORTED ACTIVITIES

USAID FROM THE AMERICAN PEOPLE

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

A.	**Content and Quality**	
1.	Title of Event (*workshop/training/conference*): Regional Laboratory Network Training on Diagnosis of Swine Diseases	**Date** 27-31 August 2012
2.	Content was relevant , up-to-date and applicable	***poor/not useful …1 to 4…good/useful*** **1 2 3 4**
3.	What new practical skills did you learn from the workshop/training?	
4.	Balance between theory and practice? *Check here if not applicable* ☐	***Too much theory …1 to 5…too much practice*** **1 2 3 4 5**
5.	Time allocated to activities	***Not enough …1 to 4…Sufficient*** **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?	***Not at all …1 to 4…Completely*** **1 2 3 4**
7.	To what extent would you say the training/workshop/conference met defined objectives?	***Not at all …1 to 4…Completely*** **1 2 3 4**
8.	To what extent would you say the training/workshop/conference met your expectations?	***Small extent …1 to 4…Great extent*** **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.)	***poor/not useful …1 to 4…good/useful*** **1 2 3 4**
11.	Invitation *Check here if not applicable* ☐	***poor…1 to 4…good*** **1 2 3 4**
12.	Flight arrangement *Check here if not applicable* ☐	***poor …1 to 4…good*** **1 2 3 4**
13.	Airport to hotel transportation *Check here if not applicable* ☐	***poor…1 to 4…good*** **1 2 3 4**
14.	Accommodation *Check here if not applicable* ☐	***poor …1 to 4…good*** **1 2 3 4**
15.	Venue / Room Facility *Check here if not applicable* ☐	***poor/not useful …1 to 4…good/useful*** **1 2 3 4**
16.	Food and drink *Check here if not applicable* ☐	***poor/not useful …1 to 4…good/useful*** **1 2 3 4**
17	Supporting documentation and/or course materials *Check here if not applicable* ☐	***poor/not useful …1 to 4…good/useful*** **1 2 3 4**

C.	Overall assessment	
18.	General comments and your overall rating of the workshop/training/conference	***poor/not useful …1 to 4…good/useful*** **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):	