Proficiency Testing for Veterinary Diagnostic Laboratories in SAARC countries

Workshop and Training Report

21st – 26th May 2012

PD-FMD, Mukteswar, India

Australian Animal Health Laboratory

Geelong, Australia

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List of Acronyms

AAHL	Australian Animal Health Laboratory
AI	Avian Influenza
ASEAN	Association of South East Asian Nations
BSCII	Biological Safety Class 2 Cabinet
BSL	Biosecurity Level
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CT	Cycle Number Threshold
DAH	Department of Animal Health
EPT	Emerging Pandemic Threat Programme
FAO	Food and Agriculture Organization of the United Nations
FMD	Foot and Mouth Disease
HPAI	Highly Pathogenic Avian Influenza
HPEDs	Highly Pathogenic Emerging Diseases
IQC	Internal Quality Control
OIE	World Organization for Animal Health
PCR	Polymerase Chain Reaction
PPE	Personal Protective Equipment
PT	Proficiency Testing
QA	Quality Assurance
QC	Quality Control
RLDLs	Regional Leading Diagnostic Laboratories
RRL	Regional Reference Laboratory
RT-PCR	Reverse Transcription Polymerase Chain Reaction
rRT-PCR	Real-time RT-PCR
SOP	Standard Operating Procedures
TADs	Transboundary Animal Diseases
USDA	United States Department of Agriculture
USAID	United States Agency for International Development
VI	Virus Isolation

1. Executive Summary

The Australian Animal Health Laboratory (AAHL) and FAO collaborated to organise laboratory training for Proficiency Testing (PT) for Veterinary Diagnostic Laboratories in SAARC countries. The training covered PT, Quality Assurance (QA) and Standardization of Diagnostic Reagents and Biosafety and was specifically designed to strengthen regional laboratory diagnostic capacity by improving QA and especially production and QA of diagnostic reagents. The training focused on three priority areas; PT, production of reagents and QA, and standardization of the reagents using foot and mouth disease (FMD) as the example. The training covered a wide range of topics including Animal Ethics, Biosafety and Biosecurity, production and analysis of proficiency testing (PT) panels and validation of reagents and tests, introducing new concepts and reinforcing previous training.

The workshop encompassed techniques and procedures that could be applied generically to the characterization of a wide range of agents. The training was focused on the national laboratories and covered the requirements under ISO17025 for QA of laboratory tests and training in providing PT under ISO 17043, to ensure laboratories are producing accurate and correct results for tests carried out in the laboratory. The training activities also represented a networking opportunity for the regional and national laboratory personnel. The training workshop programme is detailed in Annex 1.

The training covered the production of reagents from the beginning; production of antigen and inoculation of animals to produce antiserum to the testing and validation of the reagents to use for Internal Quality Control (IQC), positive and negative test controls, and for use in PT panels.

The participants produced antigen for production of antiserum and for use as positive controls in PCR and antigen detection tests. The production of antigen for inoculation into animals using adjuvant and was discussed. Animal ethics requirements were discussed and participants were introduced to Animal ethics requirements for use and handling of animals for animal experiments.

The training covered production of antiserum for use in detection and identification of agents and for serology controls. The serum collected from animals with one inoculation (agent and adjuvant), known as mono-specific or prime serum, is best used for distinguishing antigenic difference between serotypes or clades, e.g. H5 or FMD antigenic cartography. This serum is less cross reactive than antibody produced when animals are boosted with a second inoculation of live or killed virus. The boosted serum can be used for identification of agents as the cross-reactivity allows detection of all isolates e.g. all H5 clades and in serology tests as a positive control.

This training in production of QA controls used in tests (Internal Quality Control: IQC) and samples used in PT programmes covered production of FMD reagents, the techniques learnt can be applied to other agents. The purification of antigens may be required prior to production of antiserum and this was discussed. Some laboratories do not have the equipment for some of the purification procedures (e.g. ultracentrifuge purification).

The participants carried out titrations of the reagents to establish the working titres for serum samples and CT values for antigens for standardization of reagents. The participants then produced their own PT panels/IQC controls which they then tested as they would do when participating in a PT round in their laboratory.

Participants were then trained in the analysis and reporting of PT results for both PCR (genome or antigen detection) and in serology, a statistics package was also used to analyse the PT results. The statistics was used to identify random and systematic errors in the testing and included training in troubleshooting diagnostic tests using PT and IQC results. Participants were shown and practiced how to write a summary PT report and a laboratory individual report which included troubleshooting of the PT test results.

All participants were given PT results from a PT round as assignment (Annex 3). The participants then used what they had learnt in the workshop to produce a General PT report and an Individual laboratory PT report using these PT results (an example of a general and individual laboratory PT report is included in Annex 4 and 5). The participants also had to troubleshoot problems and suggest solutions in both the general and individual report.

The workshop included a general overview of the requirements of ISO17025 and Biosafety for the laboratory which included some new QA requirements specific to production of reagents and PT samples under ISO17043.

The Laboratory Training Workshop was well received by all participants and formal feedback confirmed the laboratory activities were highly successful. The participants filled out a Training Evaluation Form and Questionnaire (Annex 12), the results can be found in the individual forms which are attachments to this report and a summary is contained in this report

1.1 Recommendations:

The training should be followed up with activities to reinforce the training from the workshop. Some Regional Leading Diagnostic Laboratories (RLDLs) are involved in carrying out PT of national laboratories and/or participate in external PT, the other countries are not involved in PT. All countries have problems with having the budget to participate in PT of tests. A funded in-country follow-up activity tailored to each county's needs is recommended to make best use of the training.

The countries activities recommended can include the following activities based on the situation in each country:

- countries to produce IQC reference controls for key diseases as the first step in using the techniques learnt at this workshop for use in their laboratory and for use in all country laboratories (sub-national laboratories) using the same tests. This will allow the National laboratory to harmonise test results from all laboratories using the IQC reference controls for tests (National Reference Controls used to harmonise test results).

- countries carrying out PT testing to put in practice training knowledge through linking the national laboratory with a PT provider to provide PT to sub-national laboratories e.g. India for FMD or Pakistan for AI and NDV.
- countries put in place PT testing for key diseases e.g. India for FMD and Pakistan for H5N1 and Bangladesh for PPR.
- countries train their sub-national laboratories to produce IQC controls, laboratory controls that are standardized against National IQC controls.

Note: countries have indicated they would need funding to put in place improved QA of laboratory test and for production of reagents. The level of support will vary in each country.

Further training in PT and production of reagents is recommended to assist countries in putting in place best practice QA of laboratory tests.

2. Background

The Food and Agriculture Organization of the United Nations (FAO) is implementing an European Union (EU) funded regional project (OSRO/RAS/901/EC) entitled "Regional Cooperation Programme on Highly Pathogenic and Emerging Diseases (HPED) in South Asia" under the umbrella of the South Asian Association for Regional Cooperation (SAARC) at FAO Sub-regional ECTAD Unit in Kathmandu, Nepal. The overall objective of the project is to strengthen and empower SAARC countries in their ability to prevent, control and eradicate HPED, including HPAI, through improved veterinary and public health services and inter-sectoral collaboration on a regional basis.

The SAARC member countries have identified three Regional Leading Diagnostic Laboratories (RLDLs) for the three priority diseases in the region. These are:

- Project Directorate on Foot and Mouth Disease (PD-FMD), IVRI campus, Mukteswar - 263138 Nainital (Uttrakhand), India for foot and mouth disease (FMD)
- Virology Laboratory of Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka, Bangladesh for peste des petits ruminants (PPR)
- National Research Laboratory on Poultry Diseases (NRLPD), Islamabad, Pakistan for highly pathogenic avian influenza (HPAI)

These Laboratories are mandated to form and coordinate and lead regional network of laboratories. The regional laboratory network will maintain uniform diagnostic standards, support training of laboratory scientists/technicians and backstop regional surveillance and epidemiological studies. The networking activities that will also include proficiency testing programmes will be supported by the international OIE and FAO reference laboratories.

A number of laboratory activities have been implemented by the "Regional Cooperation Programme on Highly Pathogenic and Emerging Diseases (HPED) in South Asia" including trainings in diagnosis of FMD, need assessment of the laboratories, support to member countries to standardize diagnostic techniques for

HPAI and set up FMD virus typing facilities by the respective RLDLs. In addition the following workshops were organized. These are:

(a) A consultative workshop for establishing a network of Regional Leading Diagnostic Laboratories in South Asia was held from 2 to 4 March 2011 in Kathmandu, Nepal for establishing a network of RLDLs in South Asia. One of the important recommendations of the workshop includes developing strategy to conduct Regional Proficiency Testing.
(b) A consultative workshop on Regional Epidemiology and Laboratory networking in the SAARC region was held from 27 - 29 July 2011 in Kathmandu, Nepal.
(c) A workshop on Laboratory Information Management System (LIMS) was held in Phuket, Thailand from 8 -9 December 2011.
(d) First Laboratory Directors' Meeting and Workshop on Laboratory Networking and Proficiency Testing for Priority HPEDs in SAARC Countries from 23-24 January 2012 in Dhaka, Bangladesh. Some of the main recommendations of the workshop on Proficiency testing include: (i) the RLDLs should receive training on proficiency testing provider prior to the beginning of the proficiency testing round. (ii) proficiency testing should be organized annually for the Real time PCR and HI for HPAI; ELISA for FMD and PCR for PPR (iii) RLDLs will provide confirmation testing and carry out backstopping missions to other laboratories to address trouble shooting and provide in-house training; (iv) Quality assurance system needs to be implemented and supported. National laboratories should seek accreditation from their own country; (v) The RLDLs will supply SOPs and Regional Guiding Principles for Diagnosis of FMD, HPAI and PPR; (vi) The RLDLs should consider participating in recognized proficiency testing programme and be accredited as PT providers; and (vii) create SAARC Working Groups for Priority Diseases.

Proficiency testing (PT) forms an important part of building regional epidemiology and laboratory networks. Most laboratory accreditation bodies using ISO/IEC 17025 standard require that laboratories participate in such programmes to be accredited. These requirements emphasize the need for proficiency test providers to demonstrate their competence. Having imparted training for staff from laboratories of member states in the laboratory diagnosis of FMD and a planned training on diagnosis of PPR by RLDLs of the Regional Support Unit, it is essential to take the capacity building to the next level by building the functional laboratory networks in the region for quality management of the laboratories.

Taking on the recommendations of the workshop on proficiency testing, the Regional Support Unit, based within the FAO's Sub-regional ECTAD Unit in Kathmandu, organized the "Regional Training on Proficiency Testing for Veterinary Diagnostic Laboratories in SAARC countries" with support from the Indian Council of Agricultural Research, Government of India and Australian Animal Health Laboratory (AAHL), Geelong, Australia.

The main objectives of this training were:

i. To train RLDL staff as proficiency testing provider prior to the beginning of the proficiency testing round.
ii. To train staff of National Laboratories of the member countries in conducting and participating in proficiency testing programmes.

2.1 Expected Outcomes

i. Enhanced capacity in quality management of RLDLs
ii. The RLDLs will be able to be accredited as proficiency testing (PT) providers and coordinate the laboratory networks in a better manner by harmonizing the test results.
iii. The National laboratories will develop the capacity to participate in Proficiency testing programmes thus contributing to quality management of their laboratories

2.2 Agenda

The topics to be covered in the training included lectures and laboratory procedures, including the design and operation of PT schemes, statistical methods, reporting, and interpretation. The training programme (Annex 1) covered the requirements of the International Standards for PT through demonstration with real examples from different types of PT programmes. Techniques covered quantitative and qualitative testing and calibration.

3. Participants

A total of 22 participants from the SAARC Member States attended the proficiency testing training (Two each from RLDLs and 8 (one each) from the national laboratories and 2 from OIE laboratory for HPAI). In addition, one representative from FAO also participated. Three facilitators from AAHL facilitated the workshop, discussions on proficiency testing and training.

4. Aim:

This training programme will cover the requirements under ISO17025 for Quality Assurance (QA) of laboratory tests and training in providing Proficiency Testing (PT) to ensure laboratories are producing accurate and correct results for tests carried out in the laboratory. The training is focused on the National laboratories to train them in production of PT and QA samples/controls and in the analysis of PT results.

5. Objectives:

1. Provide training in production of QA controls used in tests (Internal Quality Control: IQC) and samples/controls used in PT programmes
    - Production of antigen for PCR and antigen detection tests
    - Production of antiserum for serology

2. Provide training in analysis and reporting of PT results and in the use of statistics.
    - How statistics can be used to identify random and systematic errors
    - Includes training in troubleshooting diagnostic tests using PT and IQC results
3. General overview of the requirements of ISO17025 and Biosafety for the laboratory.

6. Main Findings

The workshop was successful and participants reported a good understanding of PT schemes, sample production and reporting. Participants voiced their appreciation of the importance of PT as a tool for improvement of diagnostic laboratories, laboratory accreditation and staff competency.

The workshop identified that countries were all very keen to improve the quality of the tests results in their national laboratories and that it was important to then improve the tests results in sub-national laboratories. To do this countries felt they needed ongoing support to put better QA in place in the laboratory. The National laboratories felt training in production of reagents was very important and ongoing support was needed to put a regional harmonized approach in place across all countries.
The countries felt resources and funding were the major constraints in them putting in place better QA of tests through the production of QA reagents. Countries such as India and Pakistan are putting in place or wanting to put in place PT and IQC for tests and need support to ensure this is following international best practice.

The participants felt it was critical that this type of training and support continued for establishing a functional network of Regional Leading Diagnostic Laboratories in South Asia which includes the national laboratories from each country.

- Regional workshops gave opportunity for networking and building relationships
- Opportunities to discuss technical issues
- The PT workshop gave hands on experience and it was important that the workshop gave opportunities to gain both knowledge and build technical capacity

6.1 Topics covered in the Training workshop:

Introduction to Proficiency Testing

- Types of proficiency testing
- Various standards and guides for PT
- National and international practices

Requirements:

- Design of PT schemes
    - Design of Panel for PT (selection, composition, arrangement, characterization of the test material; determination of homogeneity and

stability, determination of limits, pass or fail, for the entire panel, frequency of sending, standardized diagnostic reagents)
  - Choosing the test material
  - Creation of test material and panel items (discussion of the methods used to create test materials
- Personnel and equipment
- Labelling, packaging, storage, and distribution of PT materials, items and panels
- Data analysis
- Reports
- Communication with participants

Statistical and other methods

- Statistical analysis and interpretation of PT data
- Homogeneity and Stability assessment
- Determining the assigned value
- Evaluation of performance
- Use of uncertainty

Application of the analysis and determination of action criteria

- detailed discussion on the effective and appropriate analysis and selection of action criteria on a laboratory's PT results
- establishment and unambiguous documentation of criteria and actions to be taken for each type of "pass" or "fail" by the PT providers

QA

- requirements to establish a QA system in the laboratory
- requirements for QA of a laboratory Test
- management structure for QA in the laboratory

Records

- Creation of accurate, legible, indelible, complete, unambiguous, objective, secure, and retrievable records which include all individual measurement observations, including test results and records to be kept for any submission to be tested by a particular method (e.g. test worksheets), with established and recorded retention times to be able to recreate all events relating to the preparation of the test material, the panels, and the programme
- Record-keeping and archiving systems

Procedures

- documentation of required procedures described in sufficient detail (created, approved, distributed, revised, and archived according to a documented system of document control)

Biosafety and Biosecurity

- requirements to establish a Biosafety and Biosecurity in the laboratory
- requirements for Biosafety in the laboratory for handling samples and carrying out a laboratory test
- management structure for Biosafety and Biosecurity in the laboratory

6.2 Feedback on the Proficiency Testing (PT) Assignments from the participants

All participants were given PT results from a PT round for AI as an exercise/assignment (annex 3). The exercise gave the participants a chance to practice what they have learnt and a mechanism for the facilitators to access the understanding of the participants. The participants had used what they had learnt in the workshop to produce a General PT report and an Individual laboratory PT report using these PT results. An example of a general and an individual laboratory PT report from Bhutan and Afghanistan is included in annex 4 and 5 as a reference. The participants also had to troubleshoot problems and suggest solutions in both the general and individual report. Detailed feedback to the participants was given to each participant and the report to the participants is in annex 6 – 11. Below is a summary of the outcomes and evaluation from the assignments.

PT Reporting and Analysis participation and understanding:

- Overall there was good participation and understanding of PT workshop material by participants. Assignments returned by participants demonstrated a good understanding of both statistical analysis and manner/type of feedback needed to give to PT programme participants. A better understanding of the correlation between data, statistical analysis of assay performance is required by workshop attendees, this will enable improved comment and suggestions for improvement.

- The PD-FMD assignment demonstrated a lack of understanding by their participants and need for further training and assistance for the PD-FMD laboratory, notably PD-FMD staff also did not attend all workshop sessions, PD-FMD only submitted one of the two assignments distributed to workshop participants. This will also impact negatively on the understanding of important aspects of establishing, maintaining and reporting a PT ISO 17043 accredited scheme.

6.3 Training and workshop evaluation

Training Evaluation Form and Questionnaire (Annex 12) was given to each participant after the training and workshop. The results of the individual evaluation forms are available as attachments to this report. All the participants agreed that the training workshop was useful and were satisfied with the workshop and training organization. The general feedback included: the workshop gave the opportunity for hands on training rather than just lectures and the training gave a good introduction to the production of reagents under QA requirements and the requirements for PT and IQC. This type of training is needed to strengthen the laboratory network. The workshop was excellent and covered a large range of topics.

Due to the detailed nature of Proficiency Testing ISO 17043 guidelines and statistical analysis and reporting requirements, the participants who had a lower level understanding of the English language, had more difficulty in understanding the contents of the course, although with individual help were able to achieve a basic understanding to satisfactorily complete assignments distributed at the conclusion of

the workshop. The venue could have been larger with better access to power for PCs to help with individual assistance and easier movement around the room.

The problems which may limit the application of the techniques trained include; government or administrative issues, lack of budget or funding and lack of reagents or domestic supplier and support from laboratory management.

7. Conclusions and recommendation

The workshop agreed there was a need to have a harmonised approach to disease diagnosis and the implementation of QA in the laboratories. There is a need for harmonized protocols for diagnosis and molecular characterisation of agents in animals to be used by member countries in establishing animal diagnosis in their countries especially for priority 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 PT will lead to better diagnostic tests in the countries and countries better able to help each other.

The participants were given a large number of documents along with the workshop documents, including regional and AAHL SOPs, example of a QA manual and other QA documents, guidance to establishing country SOPs and a QA system in the laboratory. The use of regional guidelines as for established influenza for the SE Asian countries along with AAHL SOPs are useful for developing country approaches and should be made available for key diseases and made available online.

QA and Biosafety guidelines are needed for the region and continued support is needed to build capacity in QA and Biosafety.

The workshop participants agreed there were still key capacity gaps in the region for the laboratories to operate to international standard (OIE and ISO17025) which include biosafety and biosecurity, QA, budget and resources. There needs to be further support and training in all laboratories so the laboratories can implement the training received at this workshop and continue the capacity building in countries and in building a strong laboratory network for South Asia.

The training provided by this workshop is very beneficial to the individuals and the laboratories they represent but to gain maximum benefit from the training in this workshop, 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 needs to be a funded in-country activities which requires the trainee and the laboratory to use the knowledge and technologies learnt.

This workshop was attended by senior laboratory staff, it is recommended that a technical officer accompanies the laboratory manager to the workshop to take full advantage of the laboratory hands on side of the workshop Proficiency Testing under ISO 17043 and ISO17025. A large proportion of the workshop content refers directly to Proficiency Testing Sample preparation requirements. As sample preparation, testing and storage procedures of proficiency testing and IQC samples are a critical part of establishing and maintaining a Proficiency Testing Programme, attendance to

PT workshops by a staff member who routinely conduct sample preparation and runs diagnostic assays is recommended to ensure technical requirements are passed on to the staff who carry out the work in the laboratory.

Some Regional Leading Diagnostic Laboratories (RLDLs) are involved in carrying out PT of national laboratories and/or participate in external PT, the other countries are not involved in PT. All countries have problems with having the budget to participate in PT of tests. A funded in-country follow-up activity tailored to each county's needs is recommended to make best use of the training.

The countries activities recommended can include the following activities based on the situation in each country:

- countries to produce IQC reference controls for key diseases as the first step in using the techniques learnt at this workshop for use in their laboratory and for use in all country laboratories (sub-national laboratories) using the same tests. This will allow the National laboratory to harmonise test results from all laboratories using the IQC reference controls for tests (National Reference Controls used to harmonise test results).
- countries carrying out PT testing to put in practice training knowledge through linking the national laboratory with a PT provider to provide PT to sub-national laboratories e.g. India for FMD or Pakistan for AI and NDV.
- countries put in place PT testing for key diseases e.g. India for FMD and Pakistan for H5N1 and Bangladesh for PPR.
- countries train their sub-national laboratories to produce IQC controls, laboratory controls that are standardized against National IQC controls.

ANNEX 1: Programme/Daily Agenda for Regional Training on Proficiency Testing for Veterinary Diagnostic Laboratories in SAARC countries

21st - 26th May 2012

Project Directorate on FMD, Mukteswar, India

Aim:
This training programme will cover the requirements under ISO17025 for Quality Assurance (QA) of laboratory tests and training in providing Proficiency Testing (PT) to ensure laboratories are producing accurate and correct results for tests carried out in the laboratory. The training is focused on the National laboratories to train them in production of PT and QA samples/controls and in the analysis of PT results.

Objectives:

4. Provide training in production of QA controls used in tests (Internal Quality Control: IQC) and samples/controls used in PT programmes.
    - Production of antigen for PCR and antigen detection tests
    - Production of antiserum for serology

5. Provide training in analysis and reporting of PT results and in the use statistics.
    - How statistics can be used to identify random and systematic errors
    - Includes training in troubleshooting diagnostic tests using PT and IQC results
6. General overview of the requirements of ISO17025 and Biosafety for the laboratory.

Note:
Participants from each country/laboratory will work in teams for serology and PCR laboratory work. The participants will be split into two groups of eight and each group of eight split into four teams. The groups will rotate from the laboratory to lecture room following the programme.

The country team on completion of the training will implement the training in-country for PT, IQC and reagent production.

Monday (Day 1)

Time	Topic	Facilitator/Speaker	Venue
09:00	Arrival at PDFMD		
09:00	Introductions and Welcome	PDFMD FAO Chris Morrissy Participants and Facilitators	Conference Hall
09:30 - 09:50	Overview of Workshop	Chris Morrissy	Conference Hall
09:50 - 11:00	Quality Assurance (QA): Overview of requirements for ISO17025	Chris Morrissy	Conference Hall
11:00	Morning Tea		
11:30 - 12:45	Proficiency Testing (PT): Overview of requirements under ISO17025 and benefits for the laboratory	Mai Hlaing Loh	Conference Hall
12:45 - 13:45	Lunch		
13:45 - 18:00	PT for Serology: • Test serum PT panel/samples by FMD LP ELISA *Group 1 and Group 2 alternate*	Shane Riddell Chris Morrissy PD-FMD Staff	ELISA Laboratory
13:45 - 18:00	Production of IQC controls and PT samples • Requirements for QA/IQC Controls and PT samples in a test. Design of a PT panel. *Group 2 and Group alternate*	Mai Hlaing Loh Chris Morrissy	ELISA Laboratory/ conference room
18:00 – 18:15	Assessment for Day		

Tuesday (Day 2)

Time	Topic	Facilitator/Speaker	Venue
09:00	Arrival at PDFMD Review of Day 1	Chris Morrissy Shane Riddell Mai Hlaing Loh PD-FMD Staff Participants	
09:30-15:30	Complete serology testing and review results. *Group 1 and Group 2 alternate*	Shane Riddell Chris Morrissy	ELISA Laboratory/ conference room
11:00-11:30	Morning tea		
09:30-15:30	Statistics and PT • Use of Software Practical examples given for staff to work on and practice using software during downtime *Group 2 and Group 1 alternate*	Mai Hlaing Loh	ELISA Laboratory/ conference room
12:45 - 13:45	Lunch		
13:45 - 15:30	Complete serology testing and review results and Statistics and PT	Mai Hlaing Loh Chris Morrissy Shane Riddell PD-FMD Staff	ELISA Laboratory/ conference room
15:30-16:00	Afternoon tea		
16:00-18:00	Analysis of PT Results Using results from previous AI PT rounds	Mai Hlaing Loh Chris Morrissy Shane Riddell PD-FMD Staff	ELISA Laboratory/ conference room
18:00-18:15	Assessment for Day	Chris Morrissy	ELISA Laboratory/ conference room

Wednesday (Day 3)

Time	Topic	Facilitator/Speaker	Venue
09:00 – 09:15	Arrival at PD-FMD Review of Day 2	Chris Morrissy Shane Riddell Mai Hlaing Loh PD-FMD Staff	PCR Laboratory/ conference room
09:30-17:30	Test antigen PT samples by FMD Realtime PCR. *Group 1 and Group 2 alternate*	Mai Hlaing Loh Chris Morrissy Shane Riddell	PCR Laboratory/ conference room
09:30-17:30	Analysis of Workshop Serology PT Results using PT software *Group 2 and Group 1 alternate*	Participants	PCR Laboratory/ conference room
11:00-11:30	Morning tea		
11:30-12:45	Test antigen PT samples by FMD Realtime PCR and Analysis of Serology PT Results (cont)	Mai Hlaing Loh Chris Morrissy Shane Riddell	ELISA and PCR Laboratory/ conference room
12:45 - 13:45	Lunch		
13:45-16:00	Test antigen PT samples by FMD Realtime PCR and Analysis of Serology PT Results (cont)	Mai Hlaing Loh Chris Morrissy Shane Riddell	ELISA and PCR Laboratory/ conference room
16:00-16:30	Afternoon tea		
16:30-17:30	Test antigen PT samples by FMD Realtime PCR and Analysis of Serology PT Results (cont)	Mai Hlaing Loh Chris Morrissy Shane Riddell	ELISA and PCR Laboratory/ conference room
17:30 - 18:00	Review PCR results.	Mai Hlaing Loh Chris Morrissy Shane Riddell	ELISA and PCR Laboratory/ conference room
18:00-18:15	Assessment for Day	Chris Morrissy	

Thursday (Day 4)

Time	Topic	Facilitator/Speaker	Venue
09:00 – 09:30	Arrival at PD-FMD Review of Day 3	Chris Morrissy Shane Riddell Mai Hlaing Loh PD-FMD Staff	Conference room
09:30 - 11:00	Analysis of PCR Workshop PT Results and Statistics • Use of Software	Participants Mai Hlaing Loh Chris Morrissy Shane Riddell	ELISA and PCR Laboratory/ conference room
11:00 - 11:30	Morning tea		
11:30 - 12:45	Analysis of PCR Workshop PT Results and Statistics (cont) • Use of Software	Participants Mai Hlaing Loh Chris Morrissy Shane Riddell	ELISA and PCR Laboratory/ conference room
12:45 - 13:45	Lunch		
13:45 -14:30	Analysis of PCR Workshop PT Results and Statistics (cont) • Use of Software	Participants Mai Hlaing Loh Chris Morrissy Shane Riddell	Conference room
14:30 - 16:00	Preparation of a laboratory report for PT • Includes trouble shooting and recommendations for correctives actions at the laboratory using PT results	Chris Morrissy Mai Hlaing Loh Shane Riddell	Conference room
16:00 – 16:30	Afternoon Tea		
16:30 – 18:00	Production of Antigen and Antiserum • Inactivation of antigen • Testing and storage of antigen and antiserum	Shane Riddell Chris Morrissy Mai Hlaing Loh PD-FMD	Conference room
18:00 – 18:15	Assessment for Day	Chris Morrissy	

Friday (Day 5)

Time	Topic	Facilitator/Speaker	Venue
09:00 – 09:30	Arrival at PD-FMD Review of Day 4	Chris Morrissy Shane Riddell Mai Hlaing Loh PD-FMD Staff	Conference room
09:30-11:00	Preparation of a laboratory report for workshop PT results • Serology • PCR	Participants Mai Hlaing Loh	Conference room
11:00 - 11:30	Morning tea		
11:30 - 13:30	Preparation of PT samples and controls • Group discussion	Chris Morrissy Mai Hlaing Loh Shane Riddell	Conference room
12:45 - 13:45	Lunch		
14:30-15:00	Report back on Group discussions	Shane Riddell	Conference room
15:00-16:00	Review Results from PT round, preparation of samples and reports.	Chris Morrissy Mai Hlaing Loh Shane Riddell	Conference room
16:00 – 16:30	Afternoon Tea		
16:30 – 17:30	Discussion on the use of PT and IQC • Regional and National		Conference room
17:30 – 18:00	Assessment for Day	Chris Morrissy	Conference room

Saturday (Day 6)

Time	Topic	Facilitator/Speaker	Venue
09:15	Arrival at PDFMD Review of Day 5	Mohinder Oberoi	Conference room
09:30-11:00	Analysis of AI PT Results and Statistics for Serology and PCR • Using Software	Participants	Conference room
11:00 - 11:30	Morning tea		
11:30 - 13:00	Preparation of a laboratory report for workshop PT results • Serology • PCR	Participants	Conference room
13:00 – 14:00	Lunch		
14:00 - 15:30	Assessment for workshop	All	Conference room
15:30 – 16:00	Afternoon Tea		
16:00 – 17:00	Tour of PDFMD and IVRI	Participants	

ANNEX 2: LIST OF PARTICIPANTS

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ANNEX 3: Avian Influenza Type A PCR PT Assignment, Mukteswar, May 2012

Proficiency Training Workshop, Mukteswar, 25 May 2012

Avian Influenza TaqMan PCR Proficiency Testing: Report assignment.
All participants are to use the below information to create 2 reports: (1) CODED overall report to distribute to all participants (Labs A to H) and (2) an individual report to **laboratory H** with specific comments on their data and specific suggestions of improvements/changes they can make to their methods/work-flow.

The two (2) reports are to be emailed to both Chris Morrissy (chris.morrisy@csiro.au) and Mai Hlaing Loh (maihlaing.loh@csiro.au) immediately upon completion on Saturday the 26th of May 2012.

The below Information should be used in your assignment
Assay: This data was produced using a TaqMan Type A PCR to detect Avian Influenza on a ABI real-time machine.

Avian Influenza Type A TaqMan PT panel

Positive below 37
Indeterminate is any result above 37 and below 45
negative is anything 45 and above

Table 1 Test panel identity for Type A Round 1

Sample	Virus ID	Dilution	Clade	Result
1	H3N8	10-7	2.1.3	Positive
2	None	N/A	-	Negative
3	H5N1	10-7	2.1.3	Positive
4	H5N1	10-6	1	Positive
5	H5N1	10-3	1	Positive
6	H5N1	10-4	1	Positive
7	H5N1	10-6	2.1.3	Positive
8	NDV	10-3	-	Negative
9	H9N2	10-5	2.3.4	Positive
10	H5N1	10-4	1	Positive

Sample aims
Samples 4 and 7 are identical to assess repeatability.
Sample 2 is a negative control to assess contamination
Sample 8 is negative for Avian Influenza Type A to assess specificity.
Sample 3 is a low positive to assess sensitivity
Sample 1, 3-7, 9 and 10 are various H Types to assess detection.

Participant Submitted Result Table

sample	Virus ID	Dilution	Expected Ct	Lab A	Lab B	Lab C	Lab D	Lab E	Lab F	Lab G	Lab H
1	H3N8	10-7	34.34	33.89	33.78	33.67	34.21	34.26	34.68	35.34	32.34
2	None	N/A	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00
3	H5N1	10-7	33.45	34.52	33.43	33.45	32.76	32.34	33.67	35.21	34.32
4	H5N1	10-6	31.25	32.57	31.21	32.78	31.56	33.78	34.98	34.67	36.78
5	H5N1	10-3	25.60	25.77	26.07	26.11	25.79	25.96	25.94	26.11	25.79
6	H5N1	10-4	28.21	27.89	28.67	27.46	29.14	27.75	28.56	27.67	27.37
7	H5N1	10-6	31.25	31.23	31.78	32.12	31.98	30.67	30.88	30.87	35.78
8	NDV	10-3	45.00	45.00	45.00	45.00	45.00	45.00	45.00	45.00	37.89
9	H9N2	10-5	33.42	33.89	34.65	33.98	32.87	33.57	33.32	34.95	35.67
10	H5N1	10-4	28.79	29.56	29.45	28.76	28.34	28.58	27.43	29.12	28.87

ANNEX 4 Workshop Assignment: General Report Bhutan and Afghanistan

General Report Bhutan and Afghanistan

Avian Influenza Type A Proficiency Test

Round 1 PCR Influenza Assessment

Report Date: May 26, 2012
Report Status: FINAL
Test Name: Avian Influenza A TaqMan PCR
Test Month and Year: April-May, 2012

1.1 Assessment Summary

Avian Influenza Type A TaqMan PCR Samples

The Influenza A TaqMan PCR panel for Round 1 consisted of 10 samples which were sent to each participating laboratory with instruction to test the samples for Influenza A using the standard diagnostic Influenza A TaqMan or conventional RT-PCR test used at the individual laboratory. The samples were identified by sample numbers only and for the purpose of this report are identified in Table 1.

Table 1 Test panel identity for Type A Round 1

Sample	Virus ID	Dilution	Clade	Result
1	H3N8	10-7	2.1.3	Positive
2	None	N/A	-	Negative
3	H5N1	10-7	2.1.3	Positive
4	H5N1	10-6	1	Positive
5	H5N1	10-3	1	Positive
6	H5N1	10-4	1	Positive
7	H5N1	10-6	2.1.3	Positive
8	NDV	10-3	-	Negative
9	H9N2	10-5	2.3.4	Positive
10	H5N1	10-4	1	Positive

Survey Aims

Samples 4 and 7 are identical to assess repeatability.
Sample 2 is a negative control to assess contamination
Sample 8 is negative for Avian Influenza Type A to assess specificity.
Sample 3 is a low positive to assess sensitivity
Sample 1, 3-7, 9 and 10 are various H Types to assess detection.

1.2 Participants

There were 8 participating laboratories in Influenza A Round 1 Proficiency testing for the Avian Influenza TaqMan PCR Proficiency Testing: Report assignment. Participating laboratories are Lab. A, Lab. B, Lab. C, Lab. D, Lab. E, Lab. F, Lab. G and Lab. H. All 8 participating laboratories submitted results for the INFLUENZA A PCR PT Round 1.

1.3 Analyses and statistics

The goal of this PT panel was to determine the performance of individual laboratories for the specific test. The results are presented as median values, and qualitative and quantitative interpretation of results as reported by participating laboratory.

Results were analysed using Youden plots and Z-score (using the median and normalised interquartile range or IQR) which are described as robust statistical methods. For each pair of results two Z-scores were obtained – a between laboratory Z-score and a within laboratory Z-score. A Youden plot data provides an idea of weather the sources of error is random, systematic or both. Participants with results that are identified by the Youden plots or Z-score analysis as outliers should review test procedures.

Molecular testing – TaqMan PCR Assay for Influenza A

Results were reported as CT values and qualitative interpretation (positive, indeterminate and negative). The ABI real-time PCR machine was used.

Influenza A

All laboratories reported all positive samples correctly for detection of Influenza A isolates. One laboratory reported sample 8 positive which was a NDV positive.

All results were analysed for the identical sample pair 6 and 10. All laboratories produced results for between-laboratory variation within the normally accepted absolute score limit of 2 indicating acceptable reproducibility for the 2 selected identical paired samples (Figure 1). The calculated with-in laboratory variations except for two laboratories produced results within the normally accepted range indicating acceptable repeatability (Figure 2). The outliers need to review their test sensitivity.

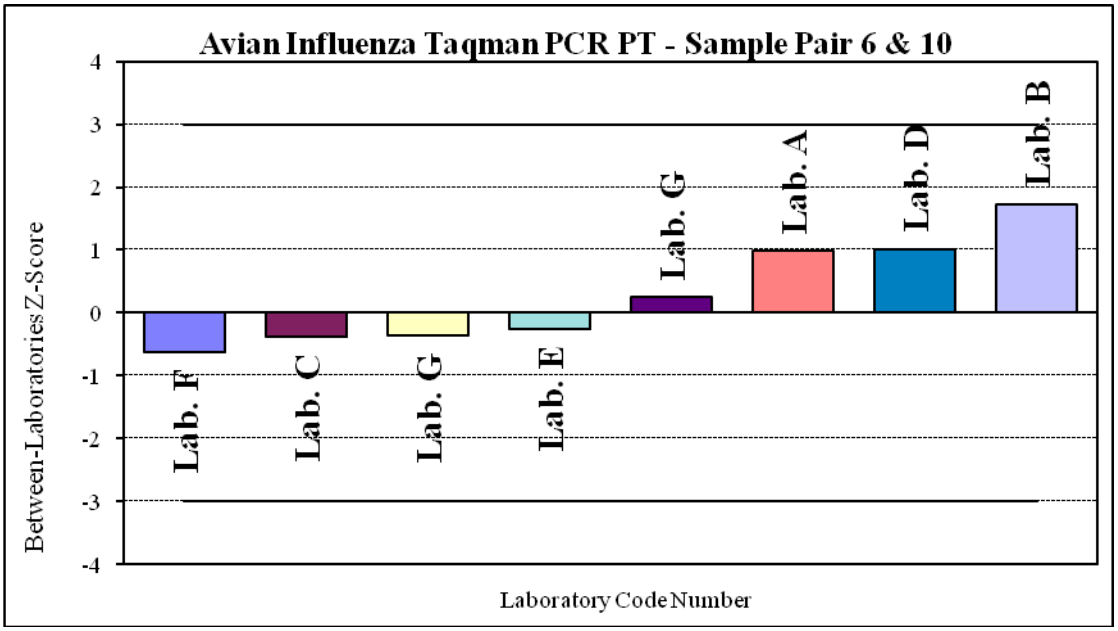

Figure 1

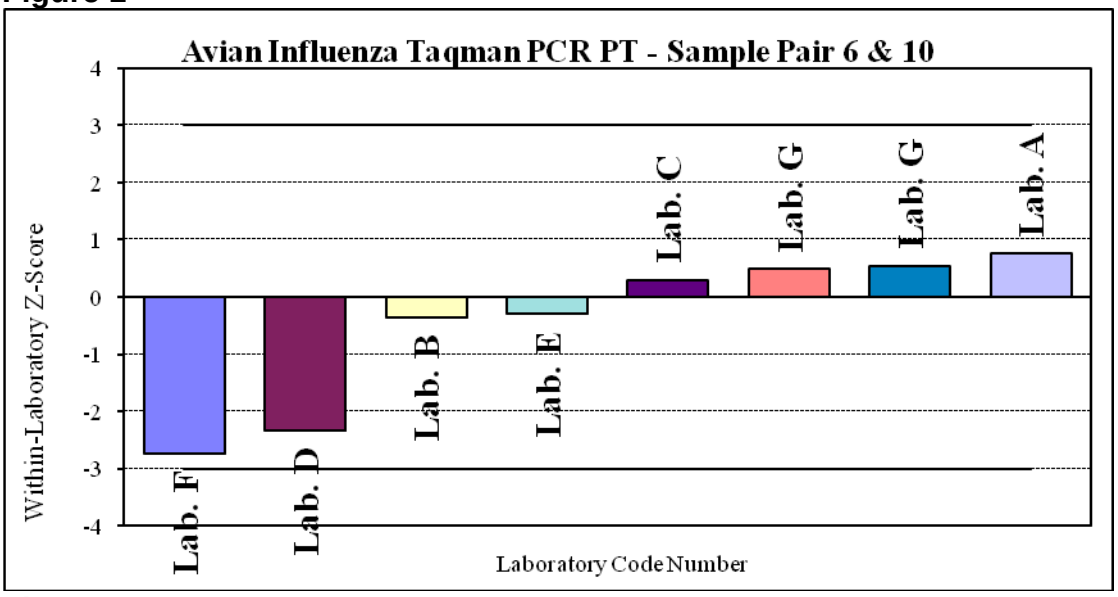

Figure 2

The Youden plot eclipse angled along the axis (Figure 3) to indicating results that have been significantly affected by random variation for one of the sample. Two laboratories fell outside the ellipse, falling in the lower right axis indicating random error. Eight laboratories performed Influenza A testing of Round 1, 7 laboratories were in agreement for all samples.

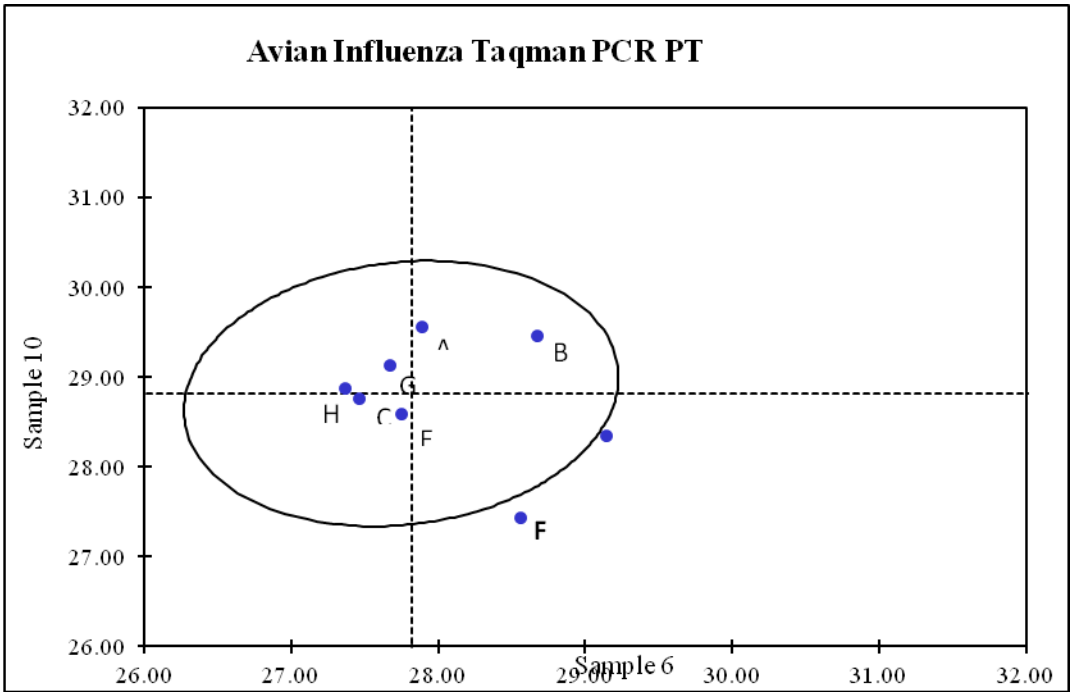

Figure 3

All laboratories had good sensitivity of their test. All laboratories had detected H types. Laboratory H needs to review the sensitivity and specificity. Laboratory H needs to retest sample 8 to check cross contamination or background (specificity). Laboratory A, C, E, F, G and H needs to review their repeatability.

We would like to thank all participants for their time and effort to test the samples and returning results on timely.

Thank you for participation in the Regional Training on Proficiency Testing for Veterinary Diagnostic Laboratories in SAARC Countries proficiency testing scheme for Molecular Diagnostics.

Further participation in PT programme would greatly benefit the laboratory's confidence in real-time PCR testing.

If you have any queries please contact our Mr. Sangay Tenzin (wamrongsangaytenzin@gmail.com)
Thank you and we look forward to your continued participation in proficiency testing.

Yours sincerely

Sangay Tenzin
Bhutan

Table 1. Qualitative interpretation of Influenza A TaqMan RT-PCR results.

sample	Virus ID	Dilution	Lab A	Lab B	Lab C	Lab D	Lab E	Lab F	Lab G	Lab H	Percent Agreement
1	H3N8	(10-7)	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100%
2	None	(N/A)	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	100%
3	H5N1	(10-7)	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100%
4	H5N1	(10-6)	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100%
5	H5N1	(10-3)	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100%
6	H5N1	(10-4)	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100%
7	H5N1	(10-6)	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100%
8	NDV	(10-3)	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Indeterminate	87.50%
9	H9N2	(10-5)	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100%
10	H5N1	(10-4)	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	100%

Table 2. Quantitative (CT) interpretation of Influenza A TaqMan RT-PCR results.

=sample	Virus ID	Dilution	Expected Ct	Lab A	Lab B	Lab C	Lab D	Lab E	Lab F	Lab G	Lab H	Median
1	H3N8	(10-7)	34.34	33.89	33.78	33.67	34.21	34.26	34.68	35.34	32.34	34.05
2	None	(N/A)	45	45	45	45	45	45	45	45	45	45
3	H5N1	(10-7)	33.45	34.52	33.43	33.45	32.76	32.34	33.67	35.21	34.32	33.56
4	H5N1	(10-6)	31.25	32.57	31.21	32.78	31.56	33.78	34.98	34.67	36.78	33.28
5	H5N1	(10-3)	25.6	25.77	26.07	26.11	25.79	25.96	25.94	26.11	25.79	25.95
6	H5N1	(10-4)	28.21	27.89	28.67	27.46	29.14	27.75	28.56	27.67	27.37	27.82
7	H5N1	(10-6)	31.25	31.23	31.78	32.12	31.98	30.67	30.88	30.87	35.78	31.505
8	NDV	(10-3)	45	45	45	45	45	45	45	45	37.89	45
9	H9N2	(10-5)	33.42	33.89	34.65	33.98	32.87	33.57	33.32	34.95	35.67	33.935
10	H5N1	(10-4)	28.79	29.56	29.45	28.76	28.34	28.58	27.43	29.12	28.87	28.815

Table 3. Influenza A PCR Assay – Summary Statistics

Statistic	Sample 6	Sample 10
No. of Results	8	8
Median	27.82	28.82
Normalised IQR	0.72	0.51
Robust CV	3%	2%
Minimum	27.37	27.43
Maximum	29.14	29.56
Range	1.77	2.13

Table 4: Influenza A PCR Assay – Within and between Laboratory Analyses

Lab Code	Between-Labs Z-Score	Lab Code	Within-Lab Z-Score
Lab. F	-0.63	Lab. F	-2.75
Lab. C	-0.38	Lab. D	-2.33
Lab. G	-0.35	Lab. B	-0.36
Lab. E	-0.25	Lab. E	-0.29
Lab. G	0.25	Lab. C	0.29
Lab. A	0.98	Lab. G	0.48
Lab. D	1.02	Lab. G	0.54
Lab. B	1.72	Lab. A	0.76

The between-laboratories and within-laboratory Z-scores are for the related pair, samples 6 and 10.

Annex 5 Workshop Assignment: Final Report to Individual laboratory Bhutan and Afghanistan

Final Report Bhutan and Afghanistan

May 2012

Director

Laboratory H

Dear Director,

Re: Regional Training on Proficiency Testing for Veterinary Diagnostic Laboratories in SAARC Countries (FAO)- PROFECIENCY TESTING ROUND 1 Final report

Thank you for participating in the proficiency testing scheme Round 1 for Influenza A real-time RT PCR. Please find attached the report for the 1st round of Proficiency Testing (PT) for Influenza A real-time RT-PCR. An explanation of your laboratory's results is presented below and further details can be found in the final coded reports. **Your results are Laboratory H**.

PT is an important part of Quality Assurance of a laboratory test to determine the performance of a test. To ensure PT results can be formally reported back to laboratories in a timely manner it is important that all laboratories return results by the due date. The laboratory is given 4 weeks to do the testing and report the results back. To carry our statistical analysis we require all laboratory PT results.

1. **Molecular Diagnostics**

Laboratory H participated in the Influenza A real-time RT PCR Proficiency Testing for Round 1. The real-time RT PCR machine used was ABI.

Influenza A real-time RT PCR

The Influenza A TaqMan PCR panel for Round 1 consisted of 10 samples which were sent to each participating laboratory with instruction to test the samples for Influenza A using the standard diagnostic Influenza A TaqMan or conventional RT-PCR test used at the individual laboratory.

All results were analysed for the identical sample pair 6 and 10. All laboratories produced results for between-laboratory variation within the normally accepted absolute score limit of 2 indicating acceptable reproducibility for the 2 selected identical paired samples. The calculated with-in laboratory variations except for two laboratories produced results within the normally accepted range indicating acceptable repeatability.

The Youden plot eclipse angled along the axis indicating results that have been significantly affected by random variation for one of the sample. Two laboratories fell outside the ellipse, falling in the lower right axis indicating random error. Eight laboratories performed Influenza A testing of Round 1, 7 laboratories were in agreement for all samples. Laboratory H detected indeterminate result for Sample 8.

Laboratory H reported 9/10 results correctly and was in agreement with the calculated median for these samples. Sample 8 was reported to be indeterminate when it was expected to be negative and needs to retest sample 8 to check specificity. Laboratory H needs to review the sensitivity and specificity as CT values for sample 4 and 6 you got are at the threshold. Laboratory H needs to review repeatability as sample 4 and 6 are identical samples.

We would like to thank all participants for their time and effort to test the samples and returning results on timely basis.

Based on the reported results we would recommend your laboratory to retest sample 8. Do you like to receive another set of PT panel for proficiency testing?

Further participation in PT programme would greatly benefit the laboratory's confidence in real-time PCR testing.

If you have any queries please contact Mr. Sangay Tenzin (wamrongsangaytenzin@gmail.com).

Thank you and we look forward to your continued participation in proficiency testing.

Yours sincerely

Sangay Tenzin
Bhutan

ANNEX 6: Feedback on Proficiency Testing Assignments: PD-FMD

Feedback on Proficiency Testing Assignments

Group: PD-FMD

Over all comment

Main Report:
Overall this report was very well written. The formatting and flow were good. However, comments on the statistical data demonstrated a lack of understanding of how the statistical analysis is correlated to the assay. Improvements could be made to formatting. More detailed expansion on analysis comment and more detailed suggestions to participants for improvement.

Individual laboratory letter:
PDFMD did not submit this assignment.

Components of PT Report Assignment	Feedback/Improvement/comment	Status
Report of Proficiency Testing for Real time PCR	Very good	✓
1. Report date – 26-05-2012	Very good	✓
2. Report status – preliminary	Very good	✓
3. Test Name – TaqMan RT PCR Results round 1 Influenza Type A	Very good	✓
4. Test month/year - 05/2012	Very good	✓
5. Assessment summary - A panel of ten samples consisting of 6 H5 subtype (2 from 2.1.3 clade, 4 from clade 1), 1 H3 subtype, 1 H9 subtype was assessed in real time TaqMan RT-PCR for specific detection of Influenza Type A infection along with 1 sample from NDV to check cross reactivity (specificity) and a negative sample. The samples 4, 5, 6 and 10 were tenfold serial dilution ranging of the same virus and evaluated for sensitivity detection. The samples were identified by sample numbers and for the purpose of this report are elucidated in Table 1.	Very good	✓

Table of PT panel sample identity and expected results	Very good	✓
6. Survey AIMS Samples 4 and 7 are identical to assess repeatability. Sample 2 is a negative control to assess contamination Sample 8 is negative for Avian Influenza Type A to assess specificity. Sample 3 is a low positive to assess sensitivity Sample 1, 3-7, 9 and 10 are various H Types to assess detection.	Very good	✓
Participants There were total 8 participating laboratories (A, B, C, D E, F, G and H) in round 1 to evaluate their efficiency of Influenza Type A detection test based on TaqMan realtime PCR chemistry.	Very good	✓
Statement of funding body: The funding body for PT testing was Food and Agriculture Organization (FAO).	Very good	✓
Summary of samples used: A panel of ten samples consisting of 6 H5 subtype (2 from 2.1.3 clade, 4 from clade 1), 1 H3 subtype, 1 H9 subtype was assessed in realtime TaqMan RT-PCR for specific detection of Influenza Type A infection along with 1 sample from NDV to check cross reactivity (specificity) and a negative sample. The samples 4, 5, 6 and 10 were tenfold serial dilution ranging of the same virus and evaluated for sensitivity detection.	Very good	✓
PT 4 and 7 are identical to assess repeatability. PT 2 is a negative control to assess contamination; PT 8 is negative for Avian Influenza Type A to assess specificity. PT 3 is a low positive to assess sensitivity. PT 1, 3-7, 9 and 10 are various H Types to assess detection.	Very good	✓
Table 2. Identity of samples used in the testing panel	Replicate of previous table, not required.	Requires improvement
Summary of the test type Used TaqMan RT-PCR assay was used for proficiency testing to diagnose the Influenza Type A infection.	Very good	✓
Quantitative results: The quantitative results were evaluated on the basis of threshold Cycle	Very good	✓

(Ct) values are listed in table 3.		
Table 3: Quantitative results for TaqMan RT-PCR for all the labs	Column required containing Median or average result	Requires improvement
Qualitative results The qualitative results were evaluated on the basis of threshold Cycle (Ct) values are listed in table 4.	Excellent	✓
Qualitative results for PCR for all the labs	Excellent	✓
Summary of overall observations: On analysis of all the PT samples tested by all the 8 laboratories an agreement of 100% was observed for all the PT samples except PT8. The intermediate value of PT8 sample by Lab H indicates artefact in reaction because controls reactions were perfect as expected. Lab E, F and G slightly deviated from the expected Ct in identical sample. This might had happened due to volumetric error.	Very good	✓
NOT INCLUDED: Graph Summary statistics	MISSING, needs to be included	Needs inclusion
Summary of 2 sets of sample comparisons and observations Statistically, the results were analysed for **PT1 and PT3**. Lab G and Lab H were the outliers as per the Youden plot indicating systematic error in lab H and random error in Lab G.	-Youden plot analysis comment is adequate; however wording could be improved, with reference to the Youden ellipse angle being the indicator for systematic variation.	Requires improvement
All the labs except Lab G, produced results for between- laboratory variation within the normally accepted absolute score limit of 3 indicating acceptable reproducibility for the 2 selected positive samples (-1.52 to 3.10) fig 1 and 2.	Slightly incorrect. Laboratory G's within-laboratory Z-score is acceptable (1.87), but their between-laboratory Z-score is 3.10. Above 3 is "unacceptable", in between 2 and 3 is "questionable".	Requires improvement
Lab G with >3 Z score (3.10) indicates significant increased sensitivity.	Incorrect. A better understanding of correlation between assay and statistical analysis needed. A higher score *may* indicate increased sensitivity depending on assay and data. In this case, the PT report writer should check the participant data, the participant data indicates that Laboratory G is	Requires improvement

	getting higher Ct value for sample 3 when compared to other participant laboratories, this actually indicates that they have a *decreased* sensitivity compared to other laboratories, which is why their between-laboratory Z-score is 3.10 = "unacceptable".	
For the Z score calculated for with-in laboratory variation, all laboratories results were also within the normally excepted range of -2.59 to 1.87 indicating acceptable repeatability.	Incorrect: The acceptable range is between 0 and ± 2. Improved wording to indicate that the RANGE of the participants within-laboratory Z-score is between -2.59 to 1.87. This range and other data should be supplied in the Summary Statistics table.	Requires improvement
The Youden eclipse with all the labs within the eclipse except LAB G and H (fig. 3) indicates systematic and random error respectively.	Good. Further comment required on shape and angle of Youden Plot e.g. "Youden plot is angled 45 degrees to the right and is rounded, indicating systematic variation influenced by random variation for participant data".	Requires improvement
Table 5. Between and within laboratory Z scores for the PT1 and PT3	Outlier symbol missing	Requires improvement
Fig. 1 Z score between lab for Sample Pair PT1 and PT3	Very good	✓
Fig.2 Z score within lab for Sample Pair PT1 and PT3	Very good	✓
Fig 3. Youden eclipse with all the laboratories	Very good	✓
Recommendations It is recommended that laboratories G and H may review their laboratory test procedures to minimize random and systematic errors respectively.	Suggestions with regard to the assay can be made as to *how* participants can assess their assay.	✓
Acknowledgements We would like to thank all participants for their time and effort to test the	Very good	✓

samples and returning results in a timely manner. We hope that results from this round can continue to improve test performance in the participating laboratories. Finally we want to acknowledge the FAO for funding and ANQAP for their kind permission to use the statistical programme and information for Z-score and Youden analysis. Should participants have any further queries, please contact Dr Aniket Sanyal (aniket.sanyal@gmail.com). Thank you.		
Contact Information Project Director, PD on FMD, Mukteswar-263138, Uttarakhand, India. Phone : +91-5942-286004 Fax : +91-5942-286307 Email: pattnaikb@gmail.com/ aniket.sanyal@gmail.com	Very good	✓
NOT INCLUDED	The report should include a “clear end” of report stating the words “END REPORT” on the last page.	Needs inclusion

Feedback on individual laboratory letter assignment

Components of Laboratory Letter written by PDFMD	Feedback/Improvement	Status
NO ASSIGNMENT SUBMITTED FOR “INDIVIDUAL LABORATORY LETTER”	Unsatisfactory	×

ANNEX 7 Feedback on Proficiency Testing Assignments from Bhopal

Feedback on Proficiency Testing Assignments

Group: Bhopal

Over all comment

Main Report:
Overall this report was very well written and demonstrated a good understanding of workshop material and Proficiency Testing scheme (including sample preparation, analysis, aims and reporting). Improvements could be made to formatting. More detailed expansion on analysis comment and more detailed suggestions to participants for improvement. Improved formatting and flow of report is required to increase the ease of understanding by the reader.

Individual laboratory letter:
Overall this individual laboratory letter is very well written and covers all of requirements. A slightly better understanding of Z-scores is required. Slightly better formatting and flow of letter is required.

Components of PT Report Assignment	Feedback/Improvement/comment	Status
Report of Proficiency Testing for Real time PCR	Very good	✓
1. **Report date** – 26-05-2012	Very good	
Report status – preliminary	Very good	✓
1. **Test Name –** TaqMan RT-PCR Results round 1 Influenza Type A	Very good	✓
1. **Test month/year** - 05/2012	Very good	✓
1. **Assessment summary** -	Very good	✓

A panel of ten samples consisting of 6 H5 subtype (2 from 2.1.3 clade, 4 from clade 1), 1 H3 subtype, 1 H9 subtype was assessed in real time TaqMan RT-PCR for specific detection of Influenza Type A infection along with 1 sample from NDV to check cross reactivity (specificity) and a negative sample. The samples 4, 5, 6 and 10 were tenfold serial dilution ranging of the same virus and evaluated for sensitivity detection. The samples were identified by sample numbers and for the purpose of this report are elucidated in Table 1.		
Table 1. Test Panel Identity for Type A Round 1	Very good	✓
Table1. Test Panel identity for Influenza Type A proficiency test round 1	Very good	✓
1. Survey AIMS Samples 4 and 7 are identical to assess repeatability Sample 2 is a negative control to assess contamination Sample 8 is negative for Avian Influenza Type A to assess specificity. Sample 3 is a low positive to assess sensitivity Sample 1, 3-7, 9 and 10 are various H Types to assess detection	Very good	✓
1. Participants There were total 8 participating laboratories (A, B, C, D E, F, G and H) in round 1 to evaluate their efficiency of Influenza Type A detection test based on TaqMan realtime PCR chemistry.	Very good	✓
1. Statement of funding body: The funding body for PT testing was Food and Agriculture Organization (FAO).	Very good	✓
1. Summary of samples used: A panel of ten samples consisting of 6 H5 subtype (2 from 2.1.3 clade, 4 from clade 1), 1 H3 subtype, 1 H9 subtype was assessed in realtime TaqMan RT-PCR for specific detection of Influenza Type A infection along with 1 sample from NDV to check cross reactivity (specificity) and a negative sample. The samples 4, 5, 6 and 10 were tenfold serial	Very good.	✓

dilution ranging of the same virus and evaluated for sensitivity detection.		
PT 4 and 7 are identical to assess repeatability. PT 2 is a negative control to assess contamination; PT 8 is negative for Avian Influenza Type A to assess specificity. PT 3 is a low positive to assess sensitivity. PT 1, 3-7, 9 and 10 are various H Types to assess detection.	Very good	✓
Table 2. Identity of samples used the testing panel.	Very good	✓
1. Summary of the test type Used TaqMan RT-PCR assay was used for proficiency testing to diagnose the Influenza Type A infection.	Very good	✓
1. Quantitative results The quantitative results were evaluated on the basis of threshold Cycle (Ct) values and are listed in table 3.	Very good	✓
Table 3. Quantitative results for TaqMan RT-PCR for all the labs	Good, a column with either the median or mean of participant data could be added	✓
2. Qualitative results The qualitative results were evaluated on the basis of threshold Cycle (Ct) values are listed in table 4.	Very good	✓
Table 3 : Qualitative results for PCR for all the labs	Very good	✓
13. Summary of overall observations: On analysis of all the PT samples tested by all the 8 laboratories an agreement of 100% was observed for all the PT samples except PT8. The intermediate value of PT8 sample by Lab H indicates artefact in reaction because control reactions were perfect as expected. Lab E, F and G slightly deviated from the expected Ct in identical sample. This might had happened due to volumetric error.	-Youden plot analysis comment is adequate; however wording could be improved, with reference to the Youden ellipse angle being the indicator for systematic variation.	Requires improvement
1. Summary of 2 sets of sample comparisons and observations Statistically, the results were analyzed for PT1 and PT3. Lab G and Lab H were the outliers as per the Youden plot indicating systematic	Slightly incorrect. Laboratory G's within-laboratory Z-score is acceptable (1.87), but their between-laboratory Z-score is 3.10. Above 3 in	Requires improvement

error in lab H and random error in Lab G.	"unacceptable", in between 2 and 3 is "questionable".	
All the labs except Lab G, produced results for between- laboratory variation within the normally accepted absolute score limit of 3 indicating acceptable reproducibility for the 2 selected positive samples (-1.52 to 3.10) fig 1 and 2.	Incorrect. A better understanding of correlation between assay and statistical analysis needed. A higher score *may* indicate increased sensitivity depending on assay and data. In this case, the PT report writer should check the participant data, the participant data indicates that Laboratory G is getting higher Ct value for sample 3 when compared to other participant laboratories, this actually indicates that they have a *decreased* sensitivity compared to other laboratories, which is why their between-laboratory Z-score is 3.10 = "unacceptable".	Requires improvement
Lab G with >3 Z score (3.10) indicates significant increased sensitivity.	Incorrect: The acceptable range is between 0 and $\pm$ 2. Improved wording to indicate that the RANGE of the participants within-laboratory Z-score is between -2.59 to 1.87. This range and other data should be supplied in the Summary Statistics table.	Requires improvement
For the Z score calculated for with-in laboratory variation, all laboratories results were also within the normally accepted range of -2.59 to 1.87 indicating acceptable repeatability.	Good. Further comment required on shape and angle of Youden Plot e.g. "Youden plot is angled 45 degrees to the right and is rounded, indicating systematic variation influenced by random variation for participant data".	Requires improvement
The Youden eclipse with all the labs within the eclipse except LAB G and H (fig. 3) indicates systematic and random error respectively.	-Youden plot analysis comment is adequate; however wording could be improved, with reference	Requires improvement

	to the Youden ellipse angle being the indicator for systematic variation.	
Table of between- and within-laboratory Z-scores	Outlier symbol missing	Requires improvement
Graph of between-laboratory Z-scores	Very good	✓
Graph of within-laboratory Z-scores	Very good	✓
Youden plot	Very good	✓
NOT INCLUDED: Graph Summary statistics	MISSING, needs to be included	Needs inclusion
1. Recommendations It is recommended that laboratories G and H may review their laboratory test procedures to minimize random and systematic errors respectively.	Very good. However the inclusion of the expected Ct value is not necessary in the qualitative table	✓
Acknowledgements We would like to thank all participants for their time and effort to test the samples and returning results in a timely manner. We hope that results from this round can continue to improve test performance in the participating laboratories.	good	✓
Finally we want to acknowledge the FAO for funding and ANQAP for their kind permission to use the statistical programme and information for Z-score and Youden analysis. Should participants have any further queries, please contact Atul Kumar Pateriya (atulpateriya@hsadl.nic.in) or Dr. Richa Sood (rsood@hsadl.nic.in)	Excellent: comments on Youden plot. -the sentence “Eight laboratories performed Influenza A testing of Round 1, 7 laboratories were in agreement for all samples “could perhaps be moved to the initial section describing overall participant result submissions.	✓
Thank you for your participation	Very good	✓
Contact Information: Joint Director, High Security Animal Disease Laboratory, IVRI, Bhopal, M.P. India, Phone : +91-755-2757542; Fax : +91-755-2758842 Email: jd.hsadl@hsadl.nic.in	Very good	✓
NOT INCLUDED	The report should include a “clear end” of report stating the words “END REPORT” on the last page.	Needs inclusion

Feedback on individual laboratory letter assignment

Components of Laboratory Letter	Feedback/Improvement	Status
FMDV Proficiency Test Provider Project directorate on Foot and Mouth Disease Mukteswar	Very good	✓
Joint Director **LAB C** **Bhopal** *Sub – Proficiency testing round1 final report.*	Very good	✓
Dear sir, Thank you for participating in the proficiency testing scheme round 1 for FMDV real time PCR test and LPBE ELISA based serology. Please find attached report of 1 round of proficiency testing for paired sample for your lab. An explanation of laboratory results is presented below.	Very good	✓
Molecular diagnostics Your laboratory participated in the FMDV realtime SYBR Green I dye based PCR assay and we are pleased to inform you that your laboratory reported all the results correctly for strong positive (PT1 and 6) and negative controls (PT5) and was an agreement with the consensus median for these samples tested. However PT3 and PT4 were reported as negative where as they were expected to be positive. These samples PT3 and PT4 were log10 dilutions of strong positive PT1. All laboratories could not detect PT4 as positive and it indicates that this sample PT4 needs review by us. However PT3 was detected positive by 50% of the laboratories which was negative in your results. These results indicate that the sensitivity of your test must be reviewed again. Your laboratory	Very good	✓

produced results for between-laboratories and within-laboratories variation within the normally accepted absolute score limit 3 indicating acceptable reproducibility and repeatability for the analyzed paired samples.		
Serology Your laboratory also participated in FMDV LPBE ELISA test for the Sero-diagnosis of Type O infection. Your laboratory reported all the results correctly with 100% agreement for all the samples within 1 dilution of consensus median for all the antigens (serotype O, A and Asia1) used in the testing PT panel. Your laboratory produced results for between-laboratories and within-laboratories variation within the normally accepted absolute score limit 3 indicating acceptable reproducibility and repeatability for the analyzed paired samples.	Very good	✓
Based on the reported results the laboratory has successfully participated in round 1 of quality management project in the proficiency testing scheme for serology and molecular diagnostics. Further participation in PT programmes would strengthen your credibility as a service provider for disease surveillance.	Very good	✓
Regards Dr. Richa Sood	Very good	✓

ANNEX 8 Feedback on Proficiency Testing Assignments from Bangladesh

Feedback on Assignment by Group "Bangladesh"

Overall comment

Main Report:
Overall this report was very well written and demonstrated a good understanding of workshop material and Proficiency Testing scheme (including sample preparation, analysis, aims and reporting). Improved formatting and flow of report is required to increase the ease of understanding by the reader.

Individual Laboratory Letter:
This letter was very well written and included all required information. Comment on laboratory performance when directly compared to other participant laboratories could be expanded.

Components of Main Proficiency Testing Report	Feedback/Improvement	Status
Report on Avian Influenza TaqMan PCR Proficiency Testing Round 1 Avian Influenza TaqMan PCR Assessment	Very good: clearly stated what Round, pathogen target and test type the report is addressing	✓
Date: 26.05.2012	Very good: clearly stated the date	✓
Test Name: *Avian Influenza TaqMan PCR*	Very good: clearly stated the test type	✓
Test Month and Year: May 2012	Very good: clearly stated the test month and year	✓
Statement of funding body: FAO-OSRO/RAS/901/EC REGIONAL COOPERATION PROGRAMME ON HIGHLY PATHOGENIC AND EMERGINING DISEASES IN SAARC	Very good: acknowledged funding body and project supporting the PT programme	✓
Assessment Summary: Avian Influenza TaqMan PCR samples The Influenza A PCR panel for round 1 consisted of 10 gamma-irradiated samples which are sent to each participating laboratory with instruction to test the inactivated avian influenza samples for influenza A using the standard diagnostic type A TaqMan RT-PCR test used at the individual laboratory. The samples were identified by sample number	Very good: - clearly described the components of the PT panel - clearly described the test types used by participants to test the PT panel.	✓

only for the purpose of the report and are identified in Table 1.		
Table of collated participant results	Very good: Included table of collated participant results	✓
Survey Aims Samples 4 and 7 are identical to assess repeatability Sample 2 is a negative control to assess contamination Sample 8 is negative for Avian Influenza Type A to assess specificity Sample 3 is a low positive to assess sensitivity Sample 1, 3-7, 9 and 10 are various H Types to assess detection	Very good: listed the aim of each sample in PT panel	✓
Participants: There were 8 participating laboratories in round 1.	Very good: described how many participants were involved in this testing round	✓
Analyses and statistics The goal of this PT panel was to determine the performance of individual laboratories for the specific test. The results are presented as median values and qualitative interpretation of results as reported by each individual lab. The results were analyzed by using Youden plot and Z-score which are described as robust statistical methods. For each pair of results two Z –score were obtained –a between laboratory Z-score and a within laboratory Z-score. Youden plot was used to illustrate the data and provides an immediate idea of the dominating sources of error in the results. Participants with results that are identified by the Youden plots or Z-score analysis as outliers should review test procedures.	Very good: described the type of statistical analysis that will be used to assess participant results.	✓
Molecular – Avian Influenza TaqMan RT-PCR Results were reported as Ct values and qualitative interpretation (positive and negative). ABI realtime PCR machine was used. All results were analysed for the split sample pair 3 and 4. All laboratories produced results for between –laboratory variation within the normally accepted absolute score limit of 3 indicating acceptable	Very good: described the data submitted by participants and	✓

reproducibility for the 2 selected positive paired samples (Table 3). For the calculated within laboratory variation all laboratories produced results within the normally accepted range indicating acceptable repeatability. The Youden plot ellipse is almost circular (Fig. 3) and slightly angled to the right which indicates slight systematic error, largely influenced by a slit in laboratory results.		
Table of collated participant qualitative results	Very good. However the inclusion of the expected Ct value is not necessary in the qualitative table	✓
All laboratories for all samples except Lab H for sample 8 are in 100% agreement.	Very good: commenting on qualitative results	✓
Table of between laboratory and within-laboratory Z-score analysis	Very good	✓
Comment on between-laboratory and within-laboratory Z-score	Very good	✓
Table of summary statistics	Very good	✓
Graph of between-laboratory Z-score	Very good	✓
Graph of within-laboratory Z-score	Very good	✓
Youden Plot	Youden plot	✓
Comment on Youden plot: “The Youden plot ellipse is almost circular (Fig. 3) and slightly angled to the right which indicates slight systematic error, largely influenced by a slit in laboratory results.”	A better understanding of types of Youden plots is required the correct comment would have been “All participants fell within the ellipse, no outliers were identified. The ellipse was almost circular, indicating equal proportions of random variation and systematic variation.”	Needs improvement
Not included	Missing a “clear “END” to the report	Was not present, needs to be added

Feedback on individual laboratory letter assignment

Components of Laboratory Letter	Feedback/Improvement	Status
Date 26.05.2012	Very good: Included date a reporting requirement	✓
Director Disease Diagnosis and Control lab in Bangladesh Dear Director,	Very good: Addressed director	✓
Re: IDENTIFY Project (OSR O/INT/902/USA) and HPED Project (OSRO/RAS/901/EC)	Very good: Stated the Project number and funding codes	✓
Thank you for participating in the IDENTIFY FAO PO269569 proficiency Testing Scheme Round 2012-1 for Avian influenza. Please find attached the report for the first round Proficiency Testing (PT) for molecular diagnosis Avian influenza. An explanatory of your laboratory‘s results is presented below and further details can be found in the final coded report. Your results are Laboratory 1.	Very good: Introduction to the report	✓
Proficiency testing is an important part of Quality Assurance of a Laboratory Test to determine the performance of a test.	Very good: - a description of the importance of the PT programme	✓
To ensure PT results can be formally reported back to laboratories in a timely manner. It is that all laboratories return results by the due date.	-A reminder of timely submissions of results – this is appropriate if any participant was late to submit results, but otherwise is not necessary.	✓
The laboratory is given 4 weeks to do the testing and report the results back. To carry out statistical analysis we require all laboratory PT results.	-A description of the time frame allowed for testing should already be stated in the PT panel information letter accompanying the samples. -This statement can be included if an explanation of	Requires improvement

	the statement is included e.g. “Laboratories are given a 4 week period to test the PT panel and submit results. Results submitted after this period may not be included in the statistical analysis. PT samples tested after this period may be subject to increased degradation and results may be adversely affected”.	
1. Molecular diagnosis	Good: stating the type of testing (e.g. molecular or serology)	✓
Your laboratory participated in the avian influenza round 1.	Very good	✓
Avian Influenza TaqMan PCR	Very good	✓
Your laboratory reported all results correctly and was in agreement with the consensus median for all samples tested for the Avian Influenza TaqMan PCR assay. Lab 1 produced results for between lab and within lab variation within the normally accepted absolute score limit of three indicating acceptable reproducibility and repeatability for the analyzed paired samples.	Very good – perhaps more comment on how the laboratory performed when directly compared to other laboratories should be included	✓
Based on the reported results the laboratory has successfully participated in round 1 of the laboratory quality management project Proficiency testing for Molecular diagnosis.	Very good	✓
Further participation in PT programmes would greatly benefit the lab confidence in real time PCR testing.	Very good: encouraging the laboratory to continue to participate in PT programmes to further improve their diagnostic standards	✓
Your lab has satisfactorily completed Round 1 of the laboratory quality management project Proficiency testing scheme for molecular PCR test for Avian Influenza.	Very good: clear statement of satisfactorily passing the PT round	✓
If you have any queries, please do not hesitate to contact.	Very good: encouragement to participants to	✓
Thank you for your support of this project and we look forward to your continued participation in Proficiency Testing.	Very Good: thanking participants for participating.	✓
Regards	Very good: including contact details, a requirement of reporting.	✓

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ANNEX 9 Feed back on Proficiency Testing Assignments from Bhutan and Afghanistan

Feedback on Proficiency Testing Assignments

Group: Bhutan and Afghanistan

Over all comment

Main Report:
Overall this report was very well written and demonstrated a good understanding of workshop material and Proficiency Testing scheme (including sample preparation, analysis, aims and reporting). Improvements could be made to formatting. More detailed expansion on analysis comment and more detailed suggestions to participants for improvement. Improved formatting and flow of report is required to increase the ease of understanding by the reader.

Individual laboratory letter:
Overall this individual laboratory letter is very well written and covers all of requirements. A slightly better understanding of Z-scores is required. Slightly better formatting and flow of letter is required.

Components of PT Report Assignment	Feedback/Improvement/comment	Status
Avian Influenza Type A Proficiency Test	Very good: clearly stated pathogen target and test type the report is addressing	✓
Round 1 PCR Influenza Assessment	Very good: clearly stated what Round, pathogen target and test type the report is addressing	
Date: 26.05.2012	Very good: clearly stated the date	✓
Report Status: FINAL	Very good: clearly stated report status	✓
Test Name: Avian Influenza A TaqMan PCR	Very good: clearly stated the test type	✓
Test Month and Year: April-May, 2012	Very good: clearly stated the test month and year	✓

NOT INCLUDED	The report should include acknowledgment of funding body and project supporting the PT programme	Needs improvement
1.1 Assessment Summary **Avian Influenza Type A TaqMan PCR Samples** The Influenza A TaqMan PCR panel for Round 1 consisted of 10 samples which were sent to each participating laboratory with instruction to test the samples for Influenza A using the standard diagnostic Influenza A TaqMan or conventional RT-PCR test used at the individual laboratory. The samples were identified by sample numbers only and for the purpose of this report are identified in Table 1.	Very good: clearly described the components of the PT panel clearly described the test types used by participants to test the PT panel.	✓
Table of PT panel identity	Good: an additional column listing proper isolate classification name should be included, e.g. “A/chicken/Indonesia/Water/1/2005 H5N1”	Needs slight improvement
Samples 4 and 7 are identical to assess repeatability Sample 2 is a negative control to assess contamination Sample 8 is negative for Avian Influenza Type A to assess specificity Sample 3 is a low positive to assess sensitivity Sample 1, 3-7, 9 and 10 are various H Types to assess detection	Good: however care must be taken to ensure correct sample number is listed for the matching aim. i. e. sample 2 and 7 were both H5N1, but they were different clades, the inclusion of the full isolate name (as above) will help to eliminate this issue.	✓
1.2 Participants There were 8 participating laboratories in Influenza A Round 1 Proficiency testing for the Avian Influenza TaqMan PCR Proficiency Testing: Report assignment. Participating laboratories are Lab. A, Lab. B, Lab. C, Lab. D, Lab. E, Lab. F, Lab. G and Lab. H. All 8 participating laboratories submitted results for the INFLUENZA A PCR PT Round 1.	Very good: described how many participants were involved in this testing round	✓
1.3 Analyses and statistics The goal of this PT panel was to determine the performance of individual laboratories for the specific test. The results are presented as median values, and qualitative and quantitative interpretation of results as	Very good: describing the goal of the PT panel and a description of the statistical analysis used to analyse the participants data.	✓

reported by participating laboratory. Results were analysed using Youden plots and Z-score (using the median and normalised interquartile range or IQR) which are described as robust statistical methods. For each pair of results two Z-scores were obtained – a between laboratory Z-score and a within laboratory Z-score. The Youden plot data provides an idea whether the sources of error is random, systematic or both. Participants with results that are identified by the Youden plots or Z-score analysis as outliers should review test procedures.		
Molecular testing – TaqMan PCR Assay for Influenza A Results were reported as CT values and qualitative interpretation (positive, indeterminate and negative). The ABI real-time PCR machine was used.	Very good: described the data submitted by participants and the equipment used by laboratories.	✓
Influenza A All laboratories reported all positive samples correctly for detection of Influenza A isolates. One laboratory reported sample 8 positive which was NDV positive. All results were analysed for the identical sample pair 6 and 10. All laboratories produced results for between-laboratory variation within the normally accepted absolute score limit of 2 indicating acceptable reproducibility for the 2 selected identical paired samples (Figure 1). The calculated with-in laboratory variations except for two laboratories produced results within the normally accepted range indicating acceptable repeatability (Figure 2). The outliers need to review their test sensitivity.	Very good: description of participant data and sample pairs analysed. -a suggested reason for incorrect results may be given in this section, or in a separate section later on in the report e.g. “one laboratory incorrectly reported sample 8 as positive, however this sample was an NDV isolate. This may be due to contamination or assay specificity issues, the laboratory should review their assay with aim to improve these variables. -comment on Z-score is good. -comment of outliers is also good.	Needs improvement
NOT INCLUDED: Graph Summary statistics	MISSING, needs to be included	Needs inclusion

Graph of between-laboratory Z-score	Very good. However the inclusion of the expected Ct value is not necessary in the qualitative table	✓
Graph of within-laboratory Z-score	good	✓
Figure 2 The Youden plot ellipse angled along the axis (Figure 3) indicating results that have been significantly affected by random variation for one of the sample. Two laboratories fell outside the ellipse, falling in the lower right axis indicating random error. Eight laboratories performed Influenza A testing of Round 1, 7 laboratories were in agreement for all samples.	Excellent: comments on Youden plot. -the sentence "Eight laboratories performed Influenza A testing of Round 1, 7 laboratories were in agreement for all samples could perhaps be moved to the initial section describing overall participant result submissions.	✓
Youden Plot	Very good	✓
All laboratories had good sensitivity of their test. All laboratories had detected H types. Laboratory H needs to review the sensitivity and specificity. Laboratory H needs to retest sample 8 to check cross contamination or background (specificity). Laboratory A, C, E, F, G and H needs to review their repeatability.	Very good	✓
We would like to thank all participants for their time and effort to test the samples and returning results on timely basis.	Very good	✓
Thank you for participation in the Regional Training on Proficiency Testing for Veterinary Diagnostic Laboratories in SAARC Countries proficiency testing scheme for Molecular Diagnostics.	Very good	✓
Further participation in PT programme would greatly benefit the laboratory's confidence in real-time PCR testing.	Very good	✓
If you have any queries please contact our Mr. Sangay Tenzin (wamrongsangaytenzin@gmail.com)	Very good: providing contact details is a requirement	✓

Thank you and we look forward to your continued participation in proficiency testing.	Very good: thanking participants for participating	✓
Yours sincerely, Sangay Tenzin Bhutan	Very good: signing of report.	✓
Table of collated participant qualitative results	Very good – A Table title should be included Minor suggestion: colour choice could be improved for easier viewing, pale blue against red background very difficult for some participants to read.	✓
Table of collated quantitative results	Very good – again, an additional column listing proper isolate classification name should be considered, e.g. "A/chicken/Indonesia/Water/1/2005 H5N1"	Needs improvement
Table of summary statistics	Very good	✓
Table of Within and between-laboratory Z score analysis	The data for the two samples chosen for analysis could be included, so that participants may view data against Z-scores in one table.	Needs improvement
The between-laboratories and within-laboratory Z-scores are for the related pair, samples 6 and 10.	Good: comment on Table of Within and between-laboratory Z score analysis.	✓
NOT INCLUDED	The report should include a "clear end" of report stating the words "END REPORT" on the last page.	Needs inclusion

Feedback on individual laboratory letter assignment

Components of Laboratory Letter written by Group 2	Feedback/Improvement	Status
May 2012	Very good: Included date a reporting requirement	✓
Laboratory H	Very good: Stated what the participant's laboratory code letter is	✓
Dear Director,	Very good: addressed the director	✓
Re: Regional Training on Proficiency Testing for Veterinary Diagnostic Laboratories in SAARC Countries (FAO)- PROFECIENCY TESTING ROUND 1 Final report	Very good: stated the funding body and project code	✓
Thank you for participating in the proficiency testing scheme Round 1 for Influenza A real-time RT PCR. Please find attached the report for the 1st round of Proficiency Testing (PT) for Influenza A real-time RT-PCR. An explanation of your laboratory's results is presented below and further details can be found in the final coded reports. **Your results are Laboratory H**.	Very good: -thanks to participants for participating. -A description/introduction to the report -clearly informed laboratory what their laboratory code is.	✓
PT is an important part of Quality Assurance of a laboratory test to determine the performance of a test.	Very good: - a description of the importance of the PT programme	✓
To ensure PT results can be formally reported back to laboratories in a timely manner it is important that all laboratories return results by the due date. The laboratory is given 4 weeks to do the testing and report the results back. To carry our statistical analysis we require all laboratory PT results.	Good: A reminder of timely submissions of results -good to remind participants of the reason of the strict due date (to enable analysis) – This is appropriate if any participant was late to submit results, but otherwise is not necessary.	✓
2. Molecular Diagnostics Laboratory H participated in the Influenza A real-time RT-PCR Proficiency Testing for Round 1. The real-time RT-PCR machine used was ABI.	Very good: -restating of laboratory code -good to restate the type of testing conducted by the laboratory, the Director this letter is sent to may not be aware on what type of PCR machine the assay is conducted on.	✓

Influenza A real-time RT PCR The Influenza A TaqMan PCR panel for Round 1 consisted of 10 samples which were sent to each participating laboratory with instruction to test the samples for Influenza A using the standard diagnostic Influenza A TaqMan or conventional RT-PCR test used at the individual laboratory.	Very good: - clearly described the components of the PT panel - clearly described the test types used by participants to test the PT panel.	✓
All results were analysed for the identical sample pair 6 and 10.	Very good: clearly stating which samples are used and what type of analysis is utilised for the report.	✓
All laboratories produced results for between-laboratory variation within the normally accepted absolute score limit of 2 indicating acceptable reproducibility for the 2 selected identical paired samples.	Needs Improvement: -the acceptable absolute score should be '3' not '2'. Between 2 and 3 would be a "questionable" Z-score. -a better understanding of Z-score required	Improvement required
The calculated with-in laboratory variations except for two laboratories produced results within the normally accepted range indicating acceptable repeatability.	Very good: a clear statement of which laboratories were within the acceptable limit and a statement highlighting good repeatability amongst participating laboratories.	✓
The Youden plot ellipse angled along the axis to indicating results that have been significantly affected by random variation for one of the sample. Two laboratories fell outside the ellipse, falling in the lower right axis indicating random error. Eight laboratories performed Influenza A testing of Round 1, 7 laboratories were in agreement for all samples. Laboratory H detected indeterminate result for Sample 8.	Excellent: comment on participant data, shows a clear understanding of the of the Youden plot. And it's associated analysis	✓
Laboratory H reported 9/10 results correctly and was in agreement with the calculated median for these samples. Sample 8 was reported to be indeterminate when it was expected to be negative and needs to retest sample 8 to check specificity.	Very good: -clearly stating how many samples out of the PT panel the participant had correctly identified.	✓
Laboratory H needs to review the sensitivity and specificity as CT values for sample 4 and 6 you got are at the threshold. Laboratory H needs to	Very good: A clear statement of how the participant's data	

review repeatability as sample 4 and 6 are identical samples.	performed in the statistical analysis. A clear suggestion of what “Laboratory H” needs to review to improve their test procedures.	
We would like to thank all participants for their time and effort to test the samples and returning results on timely.	Very good: encouraging the laboratory to continue to participate in PT programmes to further improve their diagnostic standards	✓
Based on the reported results we would recommend your laboratory to retest sample 8. Do you like to receive another set of PT panel for proficiency testing? Further participation in PT programme would greatly benefit the laboratory’s confidence in real-time PCR testing.	Very good: clear statement of satisfactorily passing the PT round	✓
If you have any queries please contact our Mr. Sangay Tenzin (wamrongsangaytenzin@gmail.com)	Good: including contact details, a requirement of reporting More contact should be included, e.g. phone, mobile phone, address of laboratory	Needs improvement
Thank you and we look forward to your continued participation in proficiency testing.	Very Good: thanking participants for participating	✓
Yours sincerely, Sangay Tenzin Bhutan	Very good: clearly stating who to contact and who issued the report.	✓

ANNEX 10 Feedback on Proficiency Testing Assignments from Hisar, India

Feedback on Proficiency Testing Assignments

Group: Hisar, India

Over all comment

Main Report:
Overall this report was very well written and demonstrated a very good understanding of workshop material and Proficiency Testing scheme (including sample preparation, analysis, aims and reporting). Improvements could be made to formatting. More detailed expansion on analysis comment and more detailed suggestions to participants for improvement. Improved formatting and flow of report is required to increase the ease of understanding by the reader.

Individual laboratory letter:
Overall this individual laboratory letter is very well written and covers all of requirements.

Components of PT Report Assignment	Feedback/Improvement/ comment	Status
Title: Avian Influenza TaqMan PCR Proficiency Testing	Very good	✓
Round 1 *Avian Influenza TaqMan PCR* Assessment	Very good	✓
Report Date: 26/5/2012	Very good	✓
Report Status: Preliminary	Very good	
Test Name: *Avian Influenza TaqMan PCR*	Very good	✓
Test Month and Year: May 2012	Very good	✓
Statement of funding body: FAO, **OSRO/RAS/901/EC REGIONAL COOPERATION PROGRAMME ON HIGHLY PATHOGENIC AND EMERGINING DISEASES IN SAARC**	Very good	✓
Assessment Summary:	Very good	✓
***Avian Influenza TaqMan PCR* samples:**	Very good	✓
The *Avian Influenza TaqMan PCR* **panel for round 1 consisted of 10 samples which were processed by each participatory group as per PT provider's instruction using Avian**	Very good	✓

Influenza TaqMan PCR. The samples were identified by sample numbers only and for the purpose of this report are identified in Table 1.		
Assay: This data was produced using a TaqMan Type A PCR to detect Avian Influenza on an ABI real-time machine.	Very good	✓
Key: of threshold cut off values for assay	Very good	✓
Table of PT panel Identification and expected results	Very good	✓
Sample aims Samples 4 and 7 are identical to assess repeatability Sample 2 is a negative control to assess contamination Sample 8 is negative for Avian Influenza Type A to assess specificity Sample 3 is a low positive to assess sensitivity Sample 1, 3-7, 9 and 10 are various H Types to assess detection	Very good	✓
Table of collated participant quantitative results	Very good	✓
Table of collated participant qualitative results	Very good	✓
Summary of overall observation: 1. *In Avian Influenza TaqMan PCR,* amplification were obtained successfully across different labs for different isolates and clades 2. As in PT 2, all laboratories could not get amplification indicating there was no contamination across the different labs 3. Similarly PT 8 was negative, all laboratories reported PT 8 as a negative except Lab H which showed intermediate Ct value this might be due to non specific background effect 4. All the Lab showed good sensitivity as amplification obtained by all Labs for PT 3 sample 5. All the Labs were successful in getting amplification in PT 1, 3-7, 9 and 10, indicating their ability to detect various H Types 6. Results of PT 4 and 7 showed good repeatability across the labs	Excellent	✓
Summary of two set of sample comparisons: **Sample no. 3 and 5:** Results were analysed using Z score and Youden plots. The results are presented as Z score for Ct value between labs and within Labs in Table 4 and chart 1 and 2. Z score values for PT 3 and 5 were less then 2 except lab G which indicated results agreed well between	Excellent	✓

laboratories except lab G which showed increased sensitivity. Z score values within lab were also <2 indicating agreement within groups. Positive Z score between laboratories were obtained in group A , F, G and H indicating these values were above median value indicating good reproducibility. Whereas negative Z score for obtained in group B, C, D and E indicating values were below median value. Positive Z score within laboratories A, F, G and H indicated laboratory sample pair is over estimated . Negative Z score within lab B, C and D indicated laboratory sample pair were under estimated. Since Negative Z score of low magnitude very near to median value indicated good repeatability in this group. Youden plots showed an ellipse with an angle 45^{o} to the left which indicates extensive random variation and little systemic variation. One laboratory, Lab G, fell outside the ellipse at the median indicating random variation.		
Table of between- and within-laboratory Z-score analysis	Very good	✓
Table of Summary Statistics	Very good	✓
Graph of Between-laboratory Z-scores	Very good	✓
Graph of within-laboratory Z-scores.	Very good	✓
Youden Plot	Very good	✓
Sample 3 and 4 Results were analyzed using Z score and Youden plots. The results are presented as Z score for Ct value between labs and within Labs in Table 6 and 7 and chart 3 and 4. Z score values for PT 3 and 4 were less then 2 which indicated results agreed well with other laboratories. Z score values within lab were also <2 indicating agreement within groups. Positive Z score between laboratories were obtained in group A , F, G and H indicating were above median value indicating good reproducibility. Whereas negative Z score for obtained between group B, C, D and E indicating values were below median value. Positive Z score within laboratories A, B, C and D indicated laboratory sample pair is over estimated. Negative Z score within lab E, F, G and H indicated laboratory sample pair were under estimated. Since Negative Z score of low magnitude very near to median value indicated good repeatability in this group. Youden plots showed an ellipse almost circular which indicates equal proportions of random variation and systemic variation. No outliers were recorded; all groups fell within the ellipse. Table of between- and within-laboratory Z-scores	Very good – wording could be slightly more simple, to enable participants to have a better understanding of the comments.	✓

Table of summary statistics	Very good	✓
Graph of between-laboratory results for sample pair 3 and 4	Very good	✓
Graph of within-laboratory results for sample pair 3 and 4	Very good	✓
Youden Plot for sample pair 3 and 4	Very good	
If you have any queries please do not hesitate to contact Amit Kanani (amit_kanani@hotmail.com). Our proficiency coordinator or Ravindra Sharma (rsharma698@gmail.com).	Very good	✓
Signature Dr. Ravindra Sharma PI on AICRP on FMD, Hisar (Haryana) Phone: +91- 9896823198, Mobile: +91-9824021874 Fax: +91 – 7926304423, Email: rsharma698@gmail.com	Very good	✓
NOT INCLUDED	The report should include a “clear end” of report stating the words “END REPORT” on the last page.	Needs inclusion

Feedback on individual laboratory letter assignment

Components of Laboratory Letter written by Hisar	Feedback/Improvement	Status
AICRP on FMD, Hisar (Haryana) Phone: +91- 9896823198 Mobile: +91-9824021874 Fax: +91 – 7926304423 Email: rsharma698@gmail.com AICRP FMD Laboratory Hisar, Haryana	Very good	✓
Director LAB H	Very good	✓
Dear Director,	Very good	✓
Re: OSRO/RAS/901/EC REGIONAL COOPERATION PROGRAMMEME ON HIGHLY PATHOGENIC AND EMERGINING DISEASES IN SAARC- Proficiency testing Round 1 Preliminary report	Very good	✓
Thank you for participating in FAO OSRO/RAS/901/EC REGIONAL COOPERATION PROGRAMMEME ON HIGHLY PATHOGENIC AND EMERGINING DISEASES IN SAARC- Proficiency testing Round 1 for *Avian Influenza TaqMan PCR* Assessment.	Very good	✓
Please find attached the report for the 1st round of Proficiency Testing (PT) for *Avian Influenza TaqMan PCR*. An explanation of your laboratory's results is presented below and further details can be found in the final coded report. Your results are laboratory H for *Avian Influenza TaqMan PCR* Assessment.	Very good	✓
Proficiency testing is an important part of Quality Assurance of a laboratory test to determine the performance of a test. To ensure PT results can be formally reported back to laboratories in a timely manner it is important that all laboratories return results by the due date. The laboratory is given 4 week to do the testing and report the results back.	Very good	✓

To carry out statistical analysis we required all laboratories PT results.		
Avian Influenza TaqMan PCR Assessment: Your laboratory participated in the *Avian Influenza TaqMan PCR* Assessment round 1. The results were analyzed for the split sample pair 3 and 5 and sample pair 3 and 4. Your laboratory reported all results correctly and was in agreement with the consensus median for all sample tested for the *Avian Influenza TaqMan PCR*. Results between laboratory and within laboratory variation is within the normally accepted absolute score limit of 2 indicate acceptable reproducibility and repeatability for the analyzed paired samples.	Very good	✓
Based on the reported results your laboratory has successfully participated in round one of the laboratory quality management project proficiency testing scheme for *Avian Influenza TaqMan PCR* Assessment.	Very good	✓
Further PARTICIPATION in PT programme would greatly benefit the laboratory confidence in *Avian Influenza TaqMan PCR*.	Very good	✓
If you have any queries please do not hesitate to contact Amit Kanani (amit_kanani@hotmail.com). Our proficiency testing coordinator are Ravindra Sharma(rsharma698@gmail.com)	Very good	✓
Thank you for your support for this project and we look forward to your continued participation in proficiency testing.	Very good	✓
Regards Signature Dr. Ravindra Sharma PI on AICRP on FMD, Hisar (Haryana) Phone: +91- 9896823198 Mobile: +91-9824021874 Fax: +91 – 7926304423 Email: rsharma698@gmail.com	Very good	✓

ANNEX 11 Feedback on Proficiency Testing Assignments from Nepal and Sri Lanka

Feedback on Proficiency Testing Assignments

Group: Nepal and Sri Lanka

Over all comment

Main Report:
Overall this report was very well written and demonstrated a good understanding of workshop material and Proficiency Testing scheme (including sample preparation, analysis, aims and reporting). Improvements in formatting should be made e.g. Figure captions should be below all Figures, Table captions should be above all tables. More detailed expansion on analysis comment and more detailed suggestions to participants for improvement. Improved flow of report is required to increase the ease of understanding by the reader e.g. group all analysis comments together either before or after all Tables and Figures. Scale of graph axes should be altered to suit data being analysed.

Individual laboratory letter:
Overall this individual laboratory letter is very well written and covers all of requirements. An increased understanding of Z-scores is required for improved commenting on statistical analysis of PT panel. Better formatting and flow of letter is required. Comment on performance through sample analysis was adequate, however after stating any incorrect results and asking the laboratory to review their test procedures an aim should be stated e.g. "...review your test procedures, with aim to decrease background and/or miss-priming...", this will better guide the participant laboratory to what aspect of their assay they should be reviewing/improving.

Components of Main Laboratory Report written by participants	Feedback/Improvement	Status
Avian Influenza TaqMan PCR Proficiency testing	Very good: Included date a reporting requirement	✓
AI PCR Round 2012-1	Very good: Addressed director	✓
Report date: 26 /05 /2012	Very good: Stated the PT panel and round identification	✓
Sponsored by: FAO	Very good	✓
Test name: AI Real Time PCR	Very good	✓

Test month and year: May 2012	-Correctly stated the inconsistency in results. -However, more informative suggestions for improvement should be made. E.g. “Your laboratory may have an issue with high background reactivity or contamination and should review test procedures to improve these variables”.	Requires improvement
Assessment Summary: Avian Influenza TaqMan PCR samples	Very good	✓
The AI PCR panel for round 2012-1 consisted of 10 samples which were conducted by 8 labs followed by standard diagnostic procedures using TaqMan Type A PCR to detect Avian Influenza on an ABI real-time machine. The samples were identified by sample numbers only and for the purpose of this report are identified in table-1.	Very good	✓
Table of sample identification	Very good	✓
Samples 4 and 7 are identical to assess repeatability. Sample 2 is a negative control to assess contamination Sample 8 is negative for Avian Influenza Type A to assess specificity. Sample 3 is a low positive to assess sensitivity Sample 1, 3-7, 9 and 10 are various H Types to assess detection.	Very good	✓
Participants: There are 8 participating labs in round 1.	Very good	
Analysis and Statistics: The aim of this PT panel was to determine the performance of the individual labs for the sensitivity tests. The results were analysed using Youden plot and Z-score.	Good– however slightly more detailed explanation of statistics used to analyse participant data should be included.	✓
Type of test used: AI TaqMan PCR	Very good	
Table 2: Qualitative results of AI TaqMan Real time PCR	Needs improvement – this table was left blank.	Requires improvement
Table of collated results from participant laboratories	Very good	✓
Key of above table: The green colour cells indicate positive results, The red colour cells are for negative results The yellow colour cell indicates intermediate	Very good	✓
The qualitative results of AI TaqMan PCR indicates that all the labs	Very good	✓

sample numbers 1,3,4,5, 6, 7, 9, 10 have CT values below 37 therefore they all are in 100% agreement with the expected result. Similarly sample number 2 has Ct value 45 therefore it is negative. For sample number 8, all labs got negative result except lab H which got intermediate result.		
Table of collated Quantitative results from participants	Very good	✓
Table 4: Sample comparisons and observations of Z-score values between samples 3 and 10	Good. Formatting could be improved.	✓
It can be seen in the table 4 that the Z score value obtained by the labs for the samples 3 and 10 revealed that between the labs all results lies between +2 to -2 Z- score range therefore the results of all the labs are satisfactory. Within laboratory results indicates that they have no significant variation from each groups. All groups were found to be within -2 to +2 Z- score range and with satisfactory result.	Very good	✓
NOT INCLUDED: Graph Summary statistics	MISSING, needs to be included	Needs inclusion
Graph: Between laboratory Z-score for sample 3 and 10	Very good	✓
Figure 1 shows that between laboratory Z score values of all the labs are within the satisfactory Z score range (-2 to +2).	Very good	✓
Graph: Within - laboratory Z-score for sample 3 and 10	Very good	✓
Figure 2 shows that within laboratory Z score vales of all the labs are within the satisfactory Z score range (-2 to +2).	Very good	✓
Figure 3: The between-laboratories and within-laboratory z-scores are for the related pair, samples x and y. § denotes an outlier, i.e. |z-score|? 3.	Very good	✓
Youden Plot	Scale of graph should be corrected to suit data being analysed.	Requires improvement
The Youden Plot (Figure 3) reveals that among the 8 labs for samples 3 and 10 the eclipse angle at 45^0 indicates systemic error for all the labs.	Good, further comment stating “no outliers were identified” could be included.	✓
Please contact us for further information and clarification:	Very good	✓
Contact No: 977-1-4372578	Very good	✓

Dr. Kanchana Jayasundara, Veterinary Research Institute, Sri Lanka Dr. V C Jha , National FMD and TADs Laboratory, Nepal	Very good – two contacts are better than one for PT reporting	✓
NOT INCLUDED	The report should include a “clear end” of report stating the words “END REPORT” on the last page.	Needs inclusion

Components of Laboratory Letter written by Group “Nepal and Sri Lanka”	Feedback/Improvement	Status
May 2012	Very good: Included date a reporting requirement	✓
The Director Lab H Dear Director,	Very good: Addressed director	✓
Re: Proficiency testing AI 2012 round 1 final report	Very good: Stated the PT panel and round identification	✓
Thank you for participating in the FAO proficiency testing scheme round 1 for AI Type A. Please find the test report for the first round of proficiency testing for molecular diagnostic for AI virus. Your lab is identified as lab H. An explanation of your laboratory result is presented below.	Very good	✓
Your lab participated in the AI type A and TaqMan real time PCR PT for round 1.	Very good	✓
Your laboratory reported all results correctly except all other participating laboratories obtained negative result for sample number 8 however your lab got intermediate result for sample number 8. Therefore, your lab should review the result of sample 8 because your CT value of sample number 8 is under the expected CT value 45.	-Correctly stated the inconsistency in results. -However, more informative suggestions for improvement should be made. E.g. “Your laboratory may have an issue with high background reactivity or contamination and should review test procedures to improve these variables”.	Requires improvement
We are happy to inform you based on the reported results; your laboratory has successfully participated in round 1 of the laboratory	Very good	✓

quality management proficiency testing for TaqMan real time PCR testing of AI Type A for all samples except sample number 8.		
If any queries regarding the report please do not hesitate to contact us.	Very good	✓
Thank you for your support for this project and we look forward to your continued participation in proficiency testing.	Very good	✓
Regards Dr. Dr. Kanchana Jayasundara, Veterinary Research Institute, Sri Lanka kanchvet@yahoo.com Dr. V C Jha , National FMD and TADs Laboratory, Nepal jhavc@hotmail.com	Very good	✓

ANNEX 12 TRAINING EVALUATION FORM AND QUESTIONNAIRE

Overall assessment of the event	**1 Poor**	**2**	**3**	**4 Excellent**	**Comments**
1. Content (Quality, Up to date, Relevant)	1	2	3	4	
2. Structure / Format (Duration, Activities)	1	2	3	4	
3. Organisation (Logistics, venue, resources, assistance)	1	2	3	4	

How would you rate the impact this event had or will have on:	**1 None**	**2**	**3**	**4 Highest**	**Comments**
4. Your technical knowledge on the subject	1	2	3	4	
5. Your professional activities	1	2	3	4	
6. Strengthen regional networks	1	2	3	4	
7. Improving the work of your department/unit	1	2	3	4	

Logistics:	**1 Poor**	**2**	**3**	**4 Excellent**	**Comments**
8. Invitation	1	2	3	4	
9. Flight arrangement	1	2	3	4	
10. Airport to hotel transportation	1	2	3	4	
11. Accommodation	1	2	3	4	
12. Weekend excursion	1	2	3	4	
13. Venue / Room Facility	1	2	3	4	
14. Food and drink	1	2	3	4	
15. Hotel to training transportation	1	2	3	4	
16. Supporting document	1	2	3	4	

Please give your opinion on each aspect of the seminar you attend (1 = Not satisfied to 4 = Fully Satisfied)					
Topic	**Content**	**Presentation**	**Practice**	**Usefulness**	**Fulfilled expectation**
17. Overview of Workshop					
18. Quality Assurance: Requirements of ISO 17025					
19. Proficiency testing: requirements under 17025					
20. Biosafety and Biosecurity presentation					
21. FMD LP ELISA practical session					
22. Review of results for LP ELISA					
23. Production of IQC controls and PT samples					
24. Statistics and PT: Using the software					
25. Analysis of PT results using Avian Influenza results					
26. Analysis of ELISA results					
27. PCR-practical session					
28. Analysis of PCR results					
29. Preparation of Reports					
30. Practical session (Saturday) PT analysis and preparation of report using data provided					

31. Daily Review					
32. Overall training programme					

33. What were the main strengths and weaknesses of this event?

34. Any comment or suggestion to improve Laboratory Network Activities?

Questionnaire **Name:**

1. **Why is it important to heat inactivate samples?**

2. **What equipment should be calibrated?**
    a. **Autoclave**
    b. **Biological Safety Cabinet class 2 (BSC2)**
    c. **Pipettes**
    d. **All of the above**

3. **What is homogeneity testing and why is it important?**

4. **What is IQC (and explain why they are important)?**

5. **What samples must be included in a PT panel?**

6. **What should be included in a PT report?**

7. **Give 2 reasons why PT testing is important?**

8. **What documents are important in Quality Assurance?**
    a. **Decontamination Standard operating procedure (SOP)**
    b. **Equipment calibration SOPs**
    c. **Training**
    d. **All of the above**

9. **Which of the below is the correct value for satisfactory Z score analysis**
    a. **-1.5 to +1.5**
    b. **-2 to +2**
    c. **0.5**
    d. **±0.2**

10. **Give 4 examples of what information should be recorded on coversheets for all of your laboratory tests?**

    a.

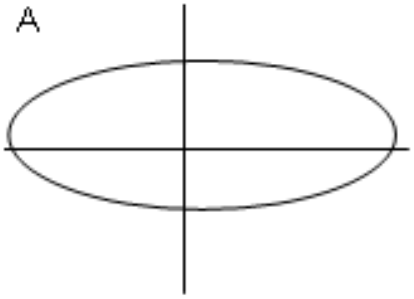
    b.

    c.

    d.

11. Which of the following diagram represents systematic error?

A



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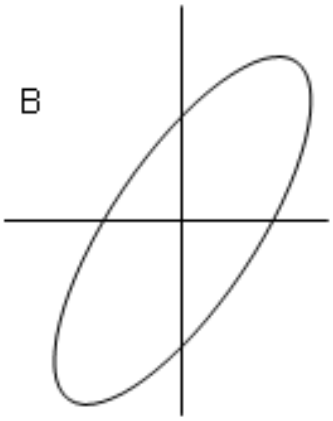

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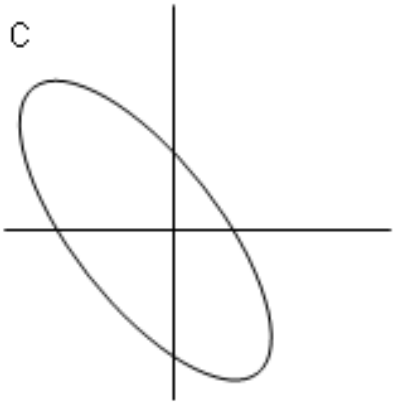

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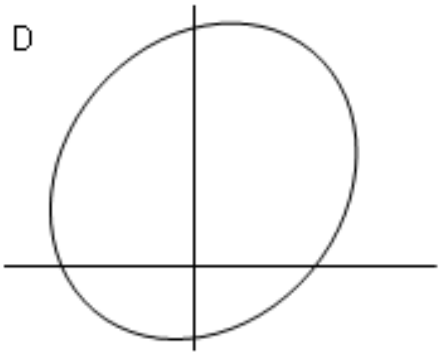

End of questionnaire

Thank you for your participation.