

Summary Report

Interlaboratory comparison testing on foot and mouth disease (FMD) ELISA typing, FMD serology by LP ELISA and NSPs test

round 1/2011 (March – August 2011)



Prepared and analyzed by
Wilai Linchongsubongkoch et al.

Regional Reference laboratory for FMD in South East Asia
Department of Livestock Development, Thailand

**Report of interlaboratory comparison testing on foot and mouth disease (FMD)
EILSA typing, FMD serology by liquid phase blocking ELISA (LP ELISA)
and non structural protein (NSPs) test**

round 1/2011 (March – August 2011)

Summary

The interlaboratory comparison exercise programme for foot and mouth disease testing round 1/2011 was conducted as a part of the 6th Meeting of SEACFMD Laboratories Network meeting, during 2-3 March 2011 at Pakchong, Thailand. In total, 17 FMD laboratories were participated, 8 FMD laboratories from South East Asia countries (Cambodia, Lao PDR, Malaysia, Myanmar, Philippines, Brunei, Vietnam (Hanoi and Ho chi Minh) and 9 Regional Veterinary Diagnostic Centers within Thailand including National Institute of Animal Health (NIAH), Bangkok and Regional Reference Laboratory for FMD in South East Asia (RRL), Pakchong. The test method used in this exercise programme consisted of antigen capture ELISA, FMD serology test by LP ELISA and NSPs test. The ELISA typing test was to be used for the detection and serotyping of FMDV, the FMD serology LP ELISA was to be used for the detection of type-specific antibody (O, A and Asia1) to FMDV structural protein and detection of antibody to non- structural protein (NSP) was by 3ABC NSP (PrioCheck) and 3B NSP (UBI). The objectives of this programme were firstly to evaluate the performance of individual operators or laboratory staff to ensure that they have competence in the assay performance on FMD diagnostic and serology test, secondly to evaluate the laboratory capability to conduct specific diagnostic test in comparing of testing results among participating laboratories by providing the ELISA reagents kit, unknown antigen samples and unknown serum samples. Participation in proficiency testing or an interlaboratory comparison programme is an essential requirement of the ISO/IEC 17025:2005 standard for laboratory testing and for veterinary testing laboratories applying for accreditation or upgrading approved tests. In this interlaboratory comparison exercise round 1/2011 has conducted during March 2011 to August 2011 and the RRL has provide all participating laboratories with reference materials, reagents, unknown samples, questionnaires, data sheets, record forms and standard operating procedures (SOPs). The test results with questionnaires and data sheet have been received from 14 participating laboratories, there were 3 laboratories have not be able to send the result back to RRL, due to an undetectable result was produced in this laboratory.

In overall, the laboratory capacity of all participating laboratories have competence in FMD serology test by LP ELISA and NSP test, just 9 laboratories have competence in FMD antigen capture and serology tests.

The detail of reagents and samples are listed as the following;

1. Questionnaire 1 set
2. Unknown antigen 10 samples
3. Unknown serum samples 5 samples
4. Reagents for LP ELISA type O, A and Asia1 which was consist of
 - 4.1 Rabbit anti FMDV type O, A, Asia1
 - 4.2 Guinea pig anti FMDV type O, A, Asia1
 - 4.3 Concentrated inactivated FMD antigen type O, A ,Asia1
5. Tracing sheet , record form and ELISA data sheet
6. SOP for ELISA typing (RRL-T-001) ,LP ELISA (RRL-T-002)
NS test (PrioCheck (RRL-T-004) and NS test (UBI) (RRL-T-003)

Please note that, the test of unknown antigen and unknown serum were requested to be performed at least 2 times, results were recorded in the data sheet forms and sent them back with questionnaires and all raw data to the RRL, Pakchong, Thailand as soon as possible.

1. The details and test results from all participating laboratories were concluded, as the follow:

Table 1. List and name of participating laboratories

Totally 17 labs were listed in this table , please note that the numbering of item is not representative the lab code no.

| Item no. * | Name/Institute | Interlaboratory sample no. | Return of results and questionnaire |
|------------|---|-------------------------------------|-------------------------------------|
| 1 | Regional Reference Laboratory for FMD in South East Asia (RRL), Pakchong, Thailand * ² | Serum no. 1-5 Antigen no.161-170 | ✓ |
| 2 | National Institute of Animal Health (NIAH), Bangkok, Thailand * ¹ | Serum no. 41-45 | ✓ |
| 3 | Regional Veterinary Diagnostic Center, Western part (Ratchaburi) , Thailand * ¹ | Serum no. 26-30 | ✓ |
| 4 | Regional Veterinary Diagnostic Center, Western part (Cholburi), Thailand * ¹ | Serum no. 11-15 | ✓ |

| Item no. | Name/Institute | Interlaboratory sample no. | Return of results and questionnaire |
|----------|---|--------------------------------------|--|
| 5 | Regional Veterinary Diagnostic Center, upper North astern part (Khonkhean), Thailand * ¹ | Serum no. 36-40 | ✓ |
| 6 | Regional Veterinary Diagnostic Center, lower North astern part (Surin), Thailand * ¹ | Serum no. 16-20 | ✓ |
| 7 | Regional Veterinary Diagnostic Center, lower Northern part (Phisanulok), Thailand * ¹ | Serum no. 21-25 | ✓ |
| 8 | Regional Veterinary Diagnostic Center, upper Northern part (Lumpang), Thailand * ² | Serum no. 6-10 Antigen no. 31-40 | ✓ |
| 9 | Regional Veterinary Diagnostic Center, Southern part (Nakonsrithammarat), Thailand * ¹ | Serum no. 31-35 | ✓ |
| 10 | FMD Laboratory, Livestock Breeding & Veterinary Department, Myanmar | Serum no. 61-65 Antigen no. 11-20 | ✓ |
| 11 | Kota Bharu Regional Veterinary Laboratory Department of Veterinary Services, Malaysia * ² | Serum no. 76-80 Antigen no. 21-30 | ✓ |
| 12 | Philippine Animal Health Center Bureau of Animal Industry, Philippines* ² | Serum no. 71-75 Antigen no. 61-70 | ✗ |
| 13 | National Veterinary Research Institute (NaVRI) Department of Animal Health and Production, Cambodia | Serum no. 61-65 Antigen no. 41-50 | ✓ |
| 14 | Center for Veterinary Diagnostics, Regional Animal Health Office, Ho Chi Minh City, Vietnam* ² | Serum no.66-70 Antigen no. 51-60 | ✓ |
| 15 | National Animal Health Center, Department of Livestock and Fisheries, Lao PDR* ² | Serum no. 71-75 Antigen no. 61-70 | ✓ |
| 16 | Department of Animal Health (DAH), National Center for veterinary Diagnosis, Hanoi, Vietnam | Serum no. 71-75 Antigen no. 81-90 | ✗ |
| 17 | Veterinary Laboratory Services, Brunei Darussalam | Serum no. 81-85 | ✗ Request for repeat testing but no result and questionnaire has submitted to RRL |

Remarks: *1 Laboratory capability of testing can be conducted only antibody detection to FMDV

* 2 Laboratory capability of testing can be conducted both antigen and antibody detection to FMDV

* item 1- 17 is not mean the Laboratory Code or Lab Code that used in plotting graph of the all figures

2. List of reagents, antigens and serum samples for FMD ELISA typing, LP ELISA and NSP test which were distributed to all participating laboratories.

Table 2. Detail of reagents and estimation working dilution use in the assay

| Item | Name/details | Batch no. | Volume | Estimate working dilution |
|--|--|------------|-------------|--|
| 1 List of reagent and unknown serum for LP ELISA and NSPs test | | | | |
| 1.1 | Rabbit anti FMDV type O189/87 | 005/2007 | 150 μ l | 1:5000 |
| 1.2 | Rabbit anti FMDV type A118/87 | 006/2007 | 150 μ l | 1:5000 |
| 1.3 | Rabbit anti FMDV type Asia1/85 | 007/2007 | 150 μ l | 1:5000 |
| 1.4 | Guinea pig anti FMDV type O189/87 | 003/2007 | 250 μ l | 1: 2000 |
| 1.5 | Guinea pig anti FMDV type A118/87 | 004/2007 | 250 μ l | 1: 1500 |
| 1.6 | Guinea pig anti FMDV type Asia1/85 | 002/2007 | 250 μ l | 1: 1000 |
| 1.7 | inactivated Concentrated FMD antigen type 189/87 | 001/2011 | 1.0 ml | 1:500 |
| 1.8 | inactivated Concentrated FMD antigen type 118/87 | 005/2010 | 1.0 ml | 1:100 |
| 1.9 | inactivated Concentrated FMD antigen type Asia1/85 | 001/2010 | 1.0 ml | 1:100 |
| 1.10 | Control C++ | 19/11/2010 | 1 ml | O = 1: 20 A = 1 :10 Asia1 = 1:15 |
| 1.11 | Control C+ | 19/11/2010 | 1 ml | O = 1:80 A = 1 : 30 Asia1 = 1: 80 |
| 1.12 | Control C- | 14/09/2010 | 1 ml | O = 1: 20 A = 1 : 20 Asia1 = 1: 20 |
| 1.13 | Unknown serum 5 sample s for LP ELISA and NSPs | No. 1-5 | 1.0 ml each | |
| 2 List of reagent and unknown antigen for ELISA typing | | | | |
| 2.1 | Control antigen for type O189/87 | 002/2009 | 0.5 ml | 1:30 |
| 2.2 | Control antigen for type A118/87 | 003/2010 | 0.5 ml | 1:10 |
| 2.3 | Control antigen for type Asia1 /85 | 002/2008 | 0.5 ml | 1:10 |
| 2.4 | Unknown antigen | No. 1-10 | 1.5 ml each | |

3. Questionnaires

All laboratories receiving and completing the questionnaires for return to the RRL provided that very useful basic information for evaluating the test results and any constraints which would affect the assay performance such as environment and laboratory condition, experience of operator, training, calibration and maintenance of equipment, water quality, raw material and chemical or electricity problem. A summary of the questionnaire follows:

3.1 Laboratories capacity

Most of the FMD testing laboratories within Thailand and in South East Asian countries indicating their high competence in FMD diagnosis either antigen detection using ELISA typing test or FMD serology using LP ELISA and NSP test. There were two sources of ELISA reagents production were recommended and used in member country laboratories, such as World Reference Laboratory for FMD, Pirbright, UK and Regional reference Laboratory for FMD, Pakchong, Thailand. As well as an harmonization of test method by ELISA typing and LP ELISA have been done and agreement have been made among member countries since the SEACFMD Lab network meeting and the OIE Reference Lab network meeting .

Antigen capture ELISA test of serotype O, A and Asia1 were the most common serotype that all country used as an basic routine typing test, there was a few additional of type C or other has been used in routine typing test such as Lab 1, Lab 11 and Lab 16 , also the RT-PCR has been introduced and now has been used as a routine FMD diagnosis in some laboratories in order to support the ELISA typing test.

3.2 Micropipettes, tips and ELISA reader

Laboratories reported the use of a range of pipette tips such as Biohit, Proline®, Micronic®, Finn tips and others. Also there were many types of single channel micropipette were used such as Finnpipette®, Biohit proline®, Eppendorf®, Thermo®, Socorex®, Gilson®, with a vary volume of 1-10 µl, 5-50 µl, 50-250 µl and 250-1000 µl.

Micropipettes for single channel and multi-channel micropipettes were mostly used with a variable volume of 5-50 µl, 50-300 µl, and Multistepper 50/100/150/200 µl.

Scientific equipment for ELISA readers was mostly from Labsystem Multiskan EX, Multiskan MS/Plus/Mx. Other brands used included Biorad, Bio-Tex, TECAN, BDSL Immunoskan and Biotech Instrument

3.3 Cleaning and maintenance of scientific equipment

Most of laboratories have set the schedule for cleaning and maintenance the

scientific equipment regularly based on the frequency of use. However, 3 laboratories (Lab 10, Lab 12 and Lab 13) do not have planned for maintenance schedule.

Recording the temperature of the essential equipment, such as incubator, refrigerator or freezer, has completely conducted by all Thailand laboratories as this is a requirement of the laboratory quality standard of ISO 17025:2005. Detail of maintenance as this follow:

- Incubator: 10 laboratories have been conducted such as Lab 1, Lab 2, lab 3, Lab 4, Lab 5, Lab 6, lab 7, Lab 8, Lab 9 and Lab 16.

- Refrigerator, Freezer : 10 laboratories have been conducted such as Lab 1, Lab 2, lab 3, Lab 4, Lab 5, Lab 6, lab 7, Lab 8, Lab 9 and Lab 16.

- Balance : 9 laboratories have been conducted such as Lab 1, Lab 2, lab 3, Lab 4, Lab 5, Lab 6, lab 7, Lab 8 and Lab 9.

- ELISA reader 10 laboratories have been conducted such as Lab 1, Lab 2, lab 3, Lab 4, Lab 5, Lab 6, lab 7, Lab 8, Lab 9 and Lab 11.

- Micropipettes 10 laboratories have been conducted such as Lab 1, Lab 2, lab 3, Lab 4, Lab 5, Lab 6, lab 7, Lab 8, Lab 9 and Lab 11.

Recording of temperature in essential equipment, it was found that all FMD laboratories within Thailand including Lab 11 and lab 16 have been done as routine recording, The other laboratories still have not implemented of temperature recording, but only conduct the regular basic cleaning of incubators, refrigerators and freezers.

3.4 Power supply and air condition system

Most laboratories indicated to have power supply problems of voltage instability or irregular supply. Therefore automatic emergency power supply or standby generators were connected with the scientific equipment such as storage refrigerators and freezers in order to keep and maintain reagents or samples in good quality. Some laboratories such as Lab 2, lab 6, Lab 10, Lab 12 and Lab 13 have not connected the scientific equipment with automatic emergency power supply for solving these problems.

All labs have air-conditioners and set room temperatures in range 24-27 °C (average temperature 25 °C) during working or testing.

3.5 Purified water production system supply to laboratory

The majority of laboratories have purified water production system or deionized water (DI) system within the institutes, except Lab 4 where a purified water production system has not yet been installed. This laboratory receives the support of purified water regularly from outside institute from Bureau of Veterinary Biologics, Pakchong , Nakhonratchasima.

3.6 Standard Operating Procedures (SOPs) of test method

Most of the laboratories within Thailand and some SEACFMD countries (Lab 10, Lab 12 and Lab 13) have used the Standard Operating Procedure prepared by the RRL as the standard method for their laboratories. There has been one laboratory (Lab 16) has used the SOP prepared by the World Reference Laboratory (WRL) Pirbright Laboratory, UK. Lab 11 has used SOPs combined between WRL, UK and Australian Animal Health Laboratory (AAHL), Australia. These SOPs for antigen typing, LP ELISA and NSP test have been harmonized among the laboratories in SEA region.

3.7 Reagents and samples storage

All laboratories have stored the reagents, serum and viral samples at -20°C . Some laboratories routinely store material at $+4^{\circ}\text{C}$, -20°C and -80°C , most using cryotube storage in the ultra-deep freezer. Some laboratories used ordinary microtube for storage at $+4^{\circ}\text{C}$ and -20°C or -40°C .

3.8 Calibration /verification of equipment

All essential equipment such as incubator, micropipette and ELISA Reader used in ELISA techniques which might affect the quality of the test result should be calibrated or verified as a regular basis. In most of laboratories, micropipettes and ELISA readers have been calibrated or verified by calibrating Institute or local company. Temperature control equipment such as the incubator, refrigerator and freezer have been calibrated annually by certified company from Siam Cement Group (SCG, Thailand) and National Food Institute. However, Lab 1, lab 2, Lab 6 and lab 9 have been conducted an in-house verification of ELISA Reader by using a standard calibrated plate as well as all micropipettes delivery volume have been verified by using the calibrated analytical balance and standard weights. Lab 11 and Lab 16 has conducted annual calibration of this essential equipment (not mentioned for the source of calibration Institute). Some laboratories (Lab 10, Lab 12 and Lab 13) have never conducted the calibration of this equipment, due to lack of supporting budget from their institutes and due to the expense required for this calibration.

3.9 Interpretation of result and determination of antibody titer

Most of laboratories read the raw data from the ELISA reader that connected directly into computer by using software program from CSIRO (which was written by Dr. Stuart Blacksell and Mr. Chris Morrissy). Recently this programme is being used in Lab 1, Lab 2, Lab 3, lab 4, Lab 5, Lab 6, Lab 7, Lab 8, Lab 9, and then transfer data into excel spread sheet written by RRL for calculating and interpreting of antibody titer.

Some laboratories have used software that comes with the ELISA reader, and then transfer data into RRL excel spread sheet for calculating the antibody titer, such as lab 10, lab 12 and lab 13.

Regarding the subject mentioned above, all participating laboratories have received training in using the RRL Excel spread sheet for the calculation of antibody titer. The technique has been transferred both within Thailand and to neighboring countries.

3.10 Establishment of internal quality control (IQC) and analysis of IQC data

All laboratories prepared a control panel set for IQC in every LP ELISA test plate consisting of strong positive control serum (C++), weak positive control serum (C+), negative control serum (C-) and antigen control (Ca). The control chart of IQC was used to evaluate daily test of IQC plate or average results from every 40 plates over the previous 2 months interval . The IQC chart is used to evaluate the trend of each control set to indicate outcome of results within the allowed acceptance limits. The IQC chart is assessed to ensure that the test has been performed correctly and is technically valid.

Suggestion and training needs from laboratory technicians

- (1) The training on quality assurance of FMD testing was requested by three laboratories (Lab 2, Lab 6 and Lab 9) in order to solve in technical problems and strengthen technical experience.
- (2) One laboratory (Lab 12) requested training on FMD diagnosis and set up of quality assurance control system regularly for antigen typing and LP ELISA test in order to support the scope and mission of the laboratory that would be worked as the national laboratory.
- (3) One laboratory (Lab 13) request for enhancing the laboratory practice on FMD diagnosis and the establishment of quality assurance control system in both antigen typing and LP ELISA typing test
- (4) Lab 10 and Lab 13 strongly request for organizing this kind of exercise such as Proficiency testing or interlaboratory comparison programme annually and they would be very grateful to be participating such a valuable FMD testing programme.

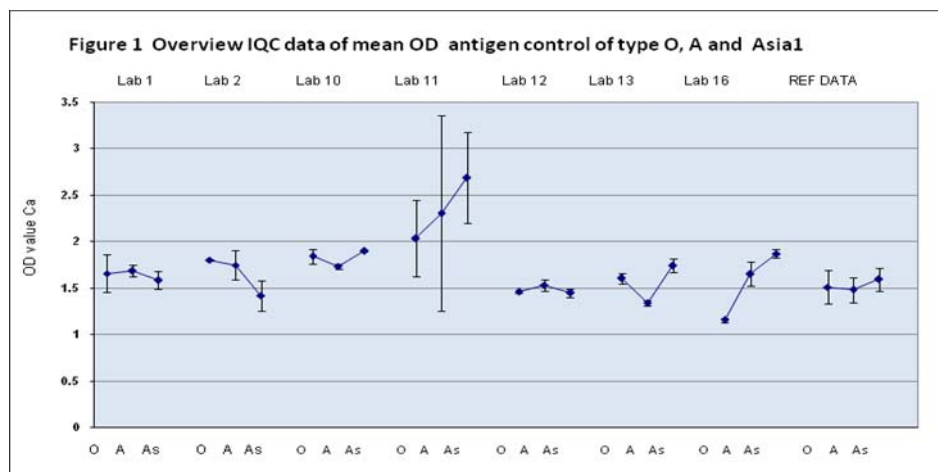
4. Summary result of ELISA typing test for serotype identification of FMDV type O, A and Asia1 are shown in table 3.

Table 3. Result of ELISA typing and OD value of sample at dilution 1:1

| Lab Code | | | Result of antigen typing test | | | | |
|---------------------|------|--------|-------------------------------|---------------------|------|--------|-----------------|
| sample no. | OD | result | Mean control antigen | sample no | OD | result | control antigen |
| Lab..... 161 | 0.01 | NVD | | Lab 81 | 0 | NVD | |
| 162 (1:10) | 1.67 | O | O = 1.66 | 82 (1:10) | 2.31 | O | O = 2.04 |
| 163 (1:10) | 0.97 | A | A = 1.68 | 83 (1:10) | 1.06 | A | A = 2.307 |
| 164 (1:4) | 1.08 | Asia1 | Asia1 = 1.58 | 84 (1:4) | 2.4 | Asia1 | Asia1 = 2.69 |
| 165 (1:4) | 1.44 | A | | 85 (1:4) | 2.07 | A | |
| 166 (1:15) | 0.76 | A | | 86 (1:15) | 0.42 | A | |
| 167 (1:15) | 0.67 | Asia1 | | 87 (1:15) | 1.31 | Asia1 | |
| 168 (1:80) | 0.63 | O | | 88 (1:80) | 0.43 | O | |
| 169 (1:10) | 0.97 | Asia1 | | 89 (1:10) | 1.3 | Asia1 | |
| 170 (1:40) | 0.79 | O | | 90 (1:40) | 0.62 | O | |
| Lab ... 31 | 0.09 | NVD | | Lab.... 181 | 0.01 | NVD | |
| 32 (1:10) | 2.09 | O | O = 1.805 | 182 (1:10) | 1.18 | O | O = 1.46 |
| 33 (1:10) | 1.45 | A | A = 1.75 | 183 (1:10) | 0.81 | A | A = 1.535 |
| 34 (1:4) | 1.83 | Asia1 | Asia1 = 1.42 | 184 (1:4) | 1.13 | Asia1 | Asia1 = 1.45 |
| 35 (1:4) | 2.02 | A | | 185 (1:4) | 1.22 | A | |
| 36 (1:15) | 0.58 | A | | 186 (1:15) | 0.85 | A | |
| 37 (1:15) | 1.84 | Asia1 | | 187 (1:15) | 0.49 | Asia1 | |
| 38 (1:80) | 0.7 | O | | 188 (1:80) | 0.24 | O | |
| 39 (1:10) | 1.4 | Asia1 | | 189 (1:10) | 0.67 | Asia1 | |
| 40 (1:40) | 1.32 | O | | 1970 (1:40) | 0.47 | O | |
| Lab... 141 | 0.01 | NVD | | Lab 11 | 0 | NVD | |
| 142 (1:10) | 2.05 | O | O = 1.845 | 12 (1:10) | 1.63 | O | O = 1.86 |
| 143 (1:10) | 1.32 | A | A = 1.74 | 13 (1:10) | 0.35 | A | A = 1.975 |
| 144 (1:4) | 2.22 | Asia1 | Asia1 = 1.905 | 14 (1:4) | 0.51 | Asia1 | Asia1 = 1.215 |
| 145 (1:4) | 2.04 | A | | 15 (1:4) | 0.92 | A | |
| 146 (1:15) | 1.07 | A | | 16 (1:15) | 0.45 | A | |
| 147 (1:15) | 1.65 | Asia1 | | 17 (1:15) | 1.09 | Asia1 | |
| 148 (1:80) | 0.91 | O | | 18 (1:80) | 0.14 | NVD | |
| 149 (1:10) | 1.79 | Asia1 | | 19 (1:10) | 0.65 | Asia1 | |
| 150 (1:40) | 1.36 | O | | 20 (1:40) | 0.35 | O | |
| Lab..... 21 | 0.12 | NVD | | REF data 1 | 0.03 | NVD | |
| 22 (1:10) | 0.72 | O | O = 1.158 | 2 (1:10) | 1.07 | O | O = 1.513 |
| 23 (1:10) | 0.46 | A | A = 1.657 | 3 (1:10) | 0.66 | A | A = 1.483 |
| 24 (1:4) | 0.87 | A | Asia1 = 1.58 | 4 (1:4) | 1.32 | Asia1 | Asia1 = 1.59 |
| 25 (1:4) | 0.77 | A | | 5 (1:4) | 0.93 | A | |
| 26 (1:15) | 0.18 | NVD | | 6 (1:15) | 0.62 | A | |
| 27 (1:15) | 0.29 | Asia1 | | 7 (1:15) | 0.74 | Asia1 | |
| 28 (1:80) | 0.16 | NVD | | 8 (1:80) | 0.36 | O | |
| 29 (1:10) | 0.21 | NVD | | 9 (1:10) | 1.05 | Asia1 | |
| 30 (1:40) | 0.13 | NVD | | 10 (1:40) | 0.65 | O | |

Remark : NDV = no virus detected

Figure 1. Overview IQC data of antigen control type O, A and Asia1 at dilution 1:1, per laboratory, an acceptance OD control should be in range 1.3-1.8



In figure 1 showed the IQC data of mean antigen control per laboratory by plotting the Mean OD \pm 1SD, in summary, most of OD antigen value was in the acceptance value in range 1.3 -1.8. One laboratory (Lab 11) gave a high OD of antigen control type O, A and Asia1 at OD 2.04, 2.307 and 2.69 respectively.

4.1 Summary and comment of the ELISA typing results on FMDV serotype identification in each laboratory and described as this following.

4.1.1 In total 10 unknown samples were distributed to 8 laboratories where the ELISA typing test have been performed, (Lab 1, lab 2, lab 10, Lab 11, lab 12, Lab 13, Lab 16 and REF. DATA) and most of laboratories gave the similar results as REF. DATA, while Lab 16 gave some different results in sample no. 26, 28, 29 and 30. In generally, these samples should report as weakly positive and no virus detected (NVD) when compared with the reference data. It would suggest that there has some error in systematic assay procedure or it necessary to check a pH condition of buffer, ELISA diluent or other non conforming work that might be happened.

4.1.2 Internal quality control (IQC), it was found that every laboratory has been set a control panel of type O, A as Asia1 in all every test plate in order to ensure the quality of test result was in technical valid and the control was in acceptance limit (OD range 1.3-1.8). In Figure 1 shown that Lab 10 obtained a high OD value of control antigen of type O and Asia1, OD at 1.845 and 1.905 respectively. In addition, Lab 11 obtained a high OD value of control antigen type O, A and Asia1 which was greater that acceptance limit, OD at 2.04, 2.307 and 2.69 respectively.

4.1.3 Total 10 unknown samples were distributed to laboratories. It found that some laboratories obtained a high OD value greater than 2.00 in the test samples. Laboratories were instructed to dilute all unknown samples 2-fold series at 1:1, 1:2, 1:4 respectively before adding to the test plate. These higher OD values might be from using the inactivated antigen without preparing working dilutions as suggested in the guidance sheet. There were other factors,

5. Summar y result of the detection of antibody to FMDV type O, A and Asia1 by LP ELISA and NS test.

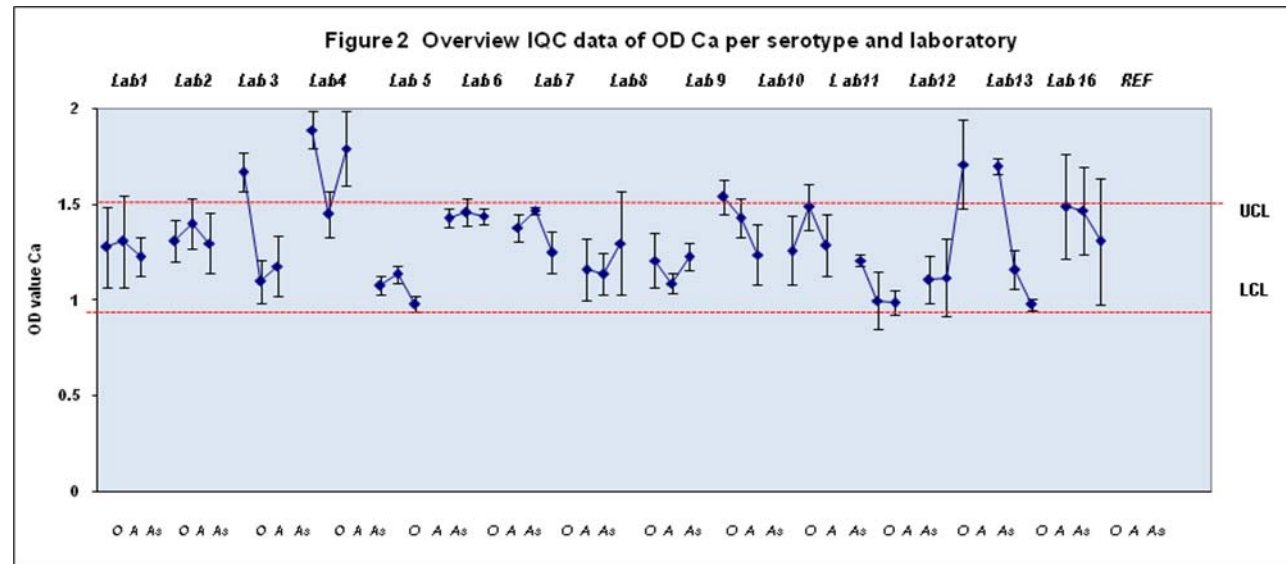
5.1 The analysis IQC data for OD antigen control (OD Ca), Percent Inhibition (%PI) of positive control serum C++, C+ and negative control serum (C-) as shown in figure 2, 3, 4 and 5 respectively

Table 4. Show the acceptance limits for IQC values of the control panel

| | LCL | UCL |
|---------|-----|-----|
| Ca (OD) | 0.9 | 1.5 |
| C++ | 90 | 100 |
| C+ | 50 | 90 |
| C- | 0 | 49 |

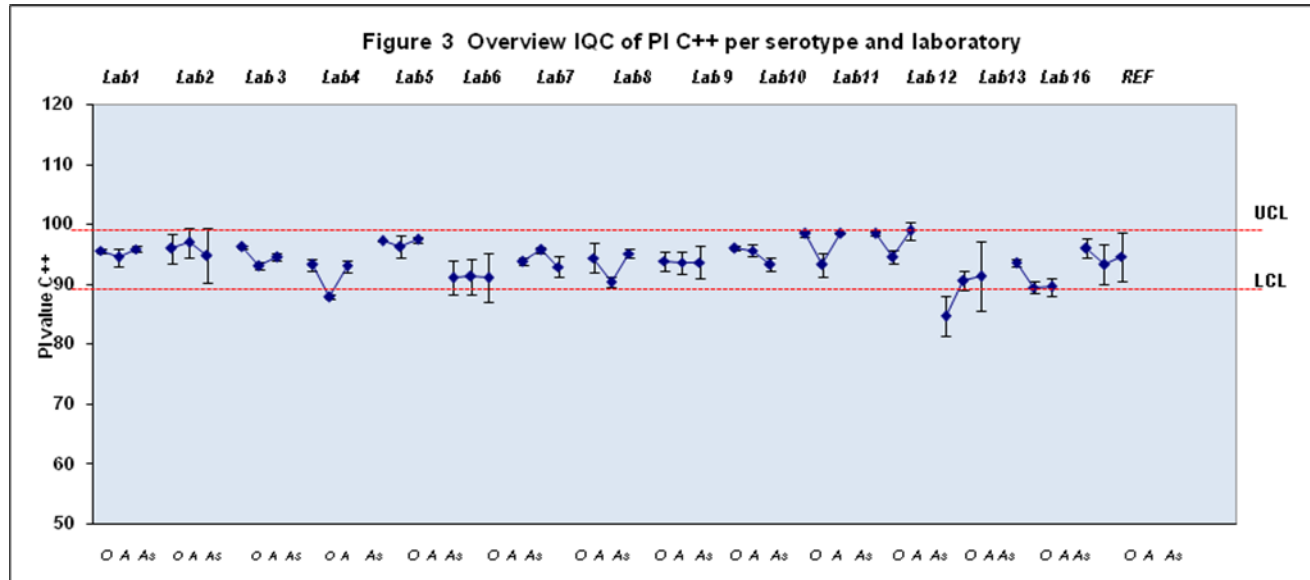
LCL = Lower control limit, UCL = Upper control limit

Figure 2. Overview IQC data of OD antigen control (OD Ca) of FMDV type O, A and Asia1



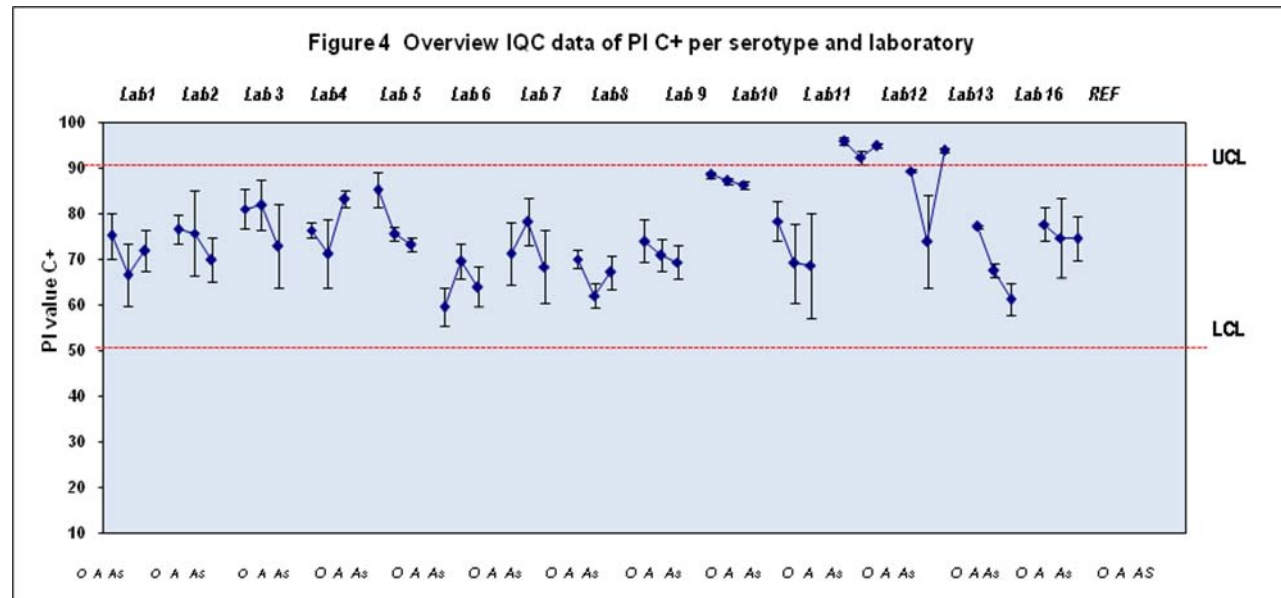
Mean OD of antigen control was shown as OD \pm 1 SD per serotype and laboratory. Most of laboratories have OD mean within the acceptance limit of OD in range 0.9-1.5. Results show that: only type O in Lab 3 gave an OD greater than upper limit, type O and Asia1 of Lab 4 gave an OD greater than upper limit, as well as type Asia 1 of Lab 12 and type O of Lab 13 gave OD greater than upper limit. This higher value might be from adjusting the working dilution without re-titration.

Figure 3. Overview IQC data of PI C++ of FMDV type O, A and Asia1 per serotype and laboratory



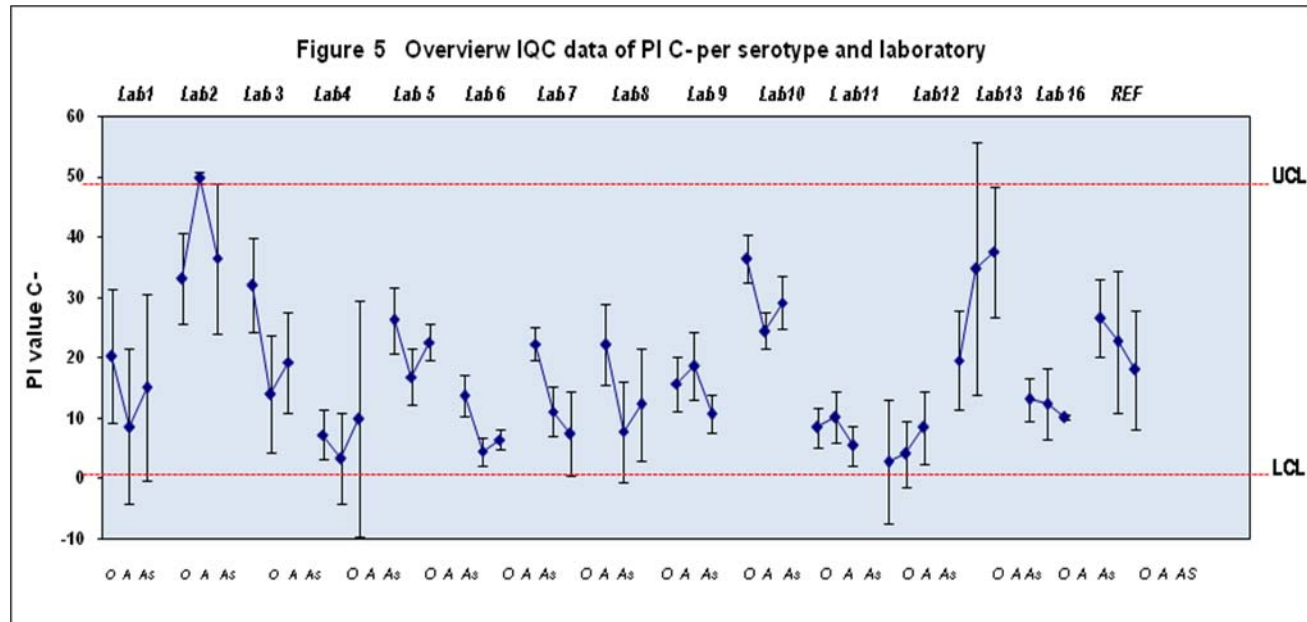
Overall, most PI C++ results were in acceptance limit in range 90-100%, exceptions was Lab 13 for which the PI C++ was below the lower acceptance limits for type O.

Figure 4. Overview IQC data of PI C+ of FMDV type O, A and Asia1 per serotype and laboratory



Overall, most PI C+ results were in acceptance limit in range 50 – 90 %. Exception was: Lab 11 for which the PI C+ of type O, A and ASIA1 were greater the upper limit; Lab 13 for which the PI C+ of type Asia1 was greater the upper limit .

Figure 5. Overview IQC data of PI C- of FMDV type O, A and Asia1 per serotype and laboratory



The PI C- of all laboratories were in acceptance limit in range 0-50%.

Figure 6. Modified Youden plot PI value of sample 3 and sample 5 FMDV type O

PI values type O for sample 3 and sample 5 from all laboratories were plotted as Youden chart between X-axis and Y-axis, then Mean PI \pm 1SD were calculated. Overall PI values of each laboratory when compared with the Reference data were shown within the \pm 1SD range of mean area, Data points for some laboratories (Lab 7, Lab 11, Lab 12 , Lab 16) were indicated on the PI plot as out of the area defined by the mean \pm 1SD.

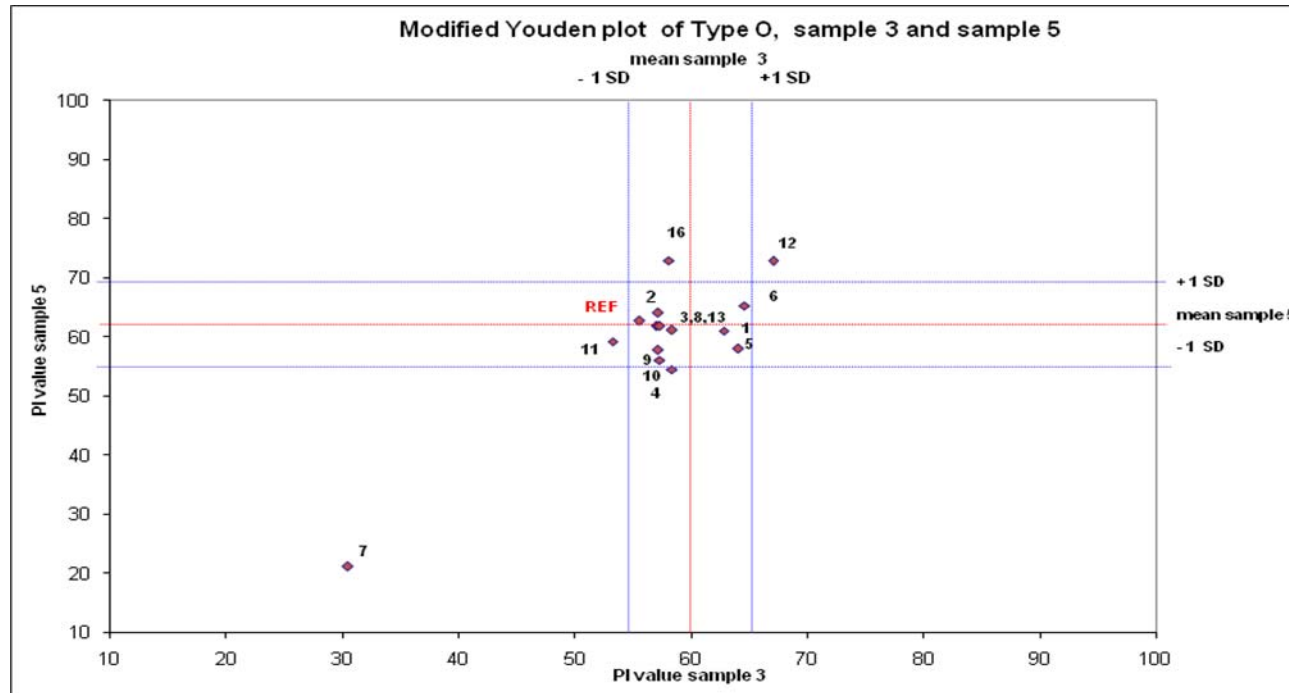


Figure 7. Modified Youden plot of PI value sample 4 and sample 2 of FMDV type A

Overall, 8 of the 14 laboratories were within the area defined by the mean \pm 1SD, while the other 4 labs (Lab 2 Lab 4, lab 11, Lab 12 and Lab 16) were indicated on the PI plot as out of the area defined by the mean \pm 1SD .

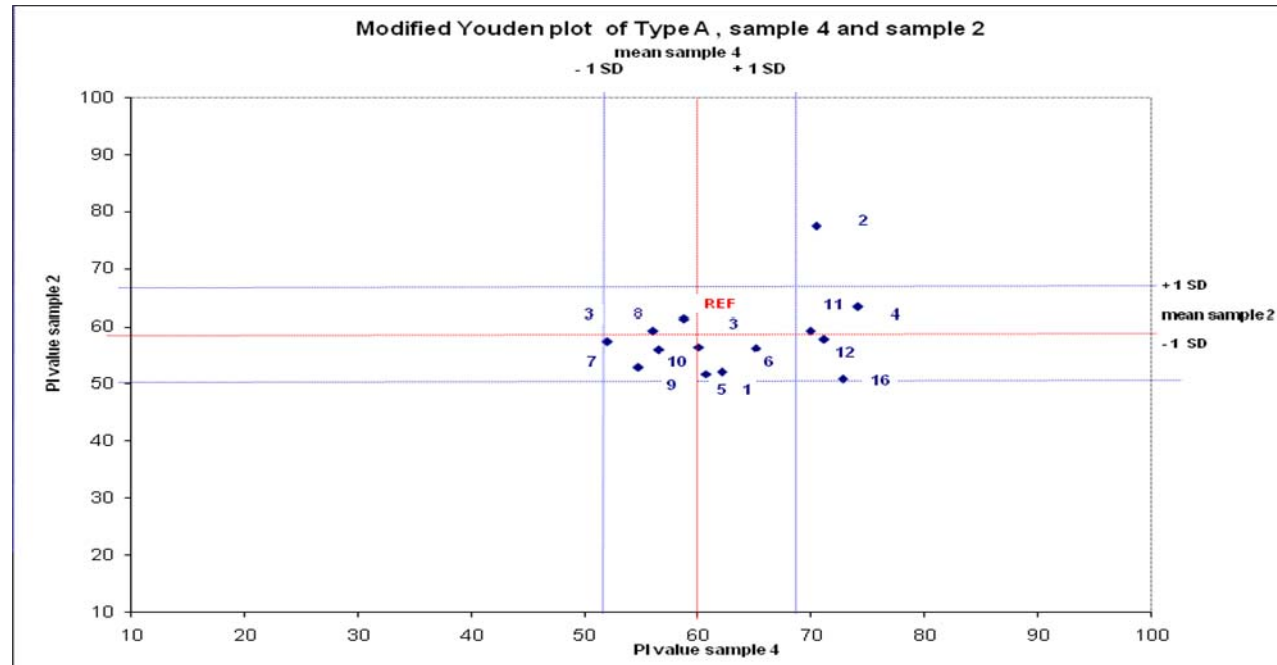


Figure 8. Modified Youden plot of PI value sample 2 and sample 4 of FMDV type Asia1

Overall most of the PI values were within the area defined by the mean \pm 1SD , while 4 labs (Lab 2, lab 4 , Lab 6 and lab 10) were defined as outside the area defined by the mean \pm 1SD

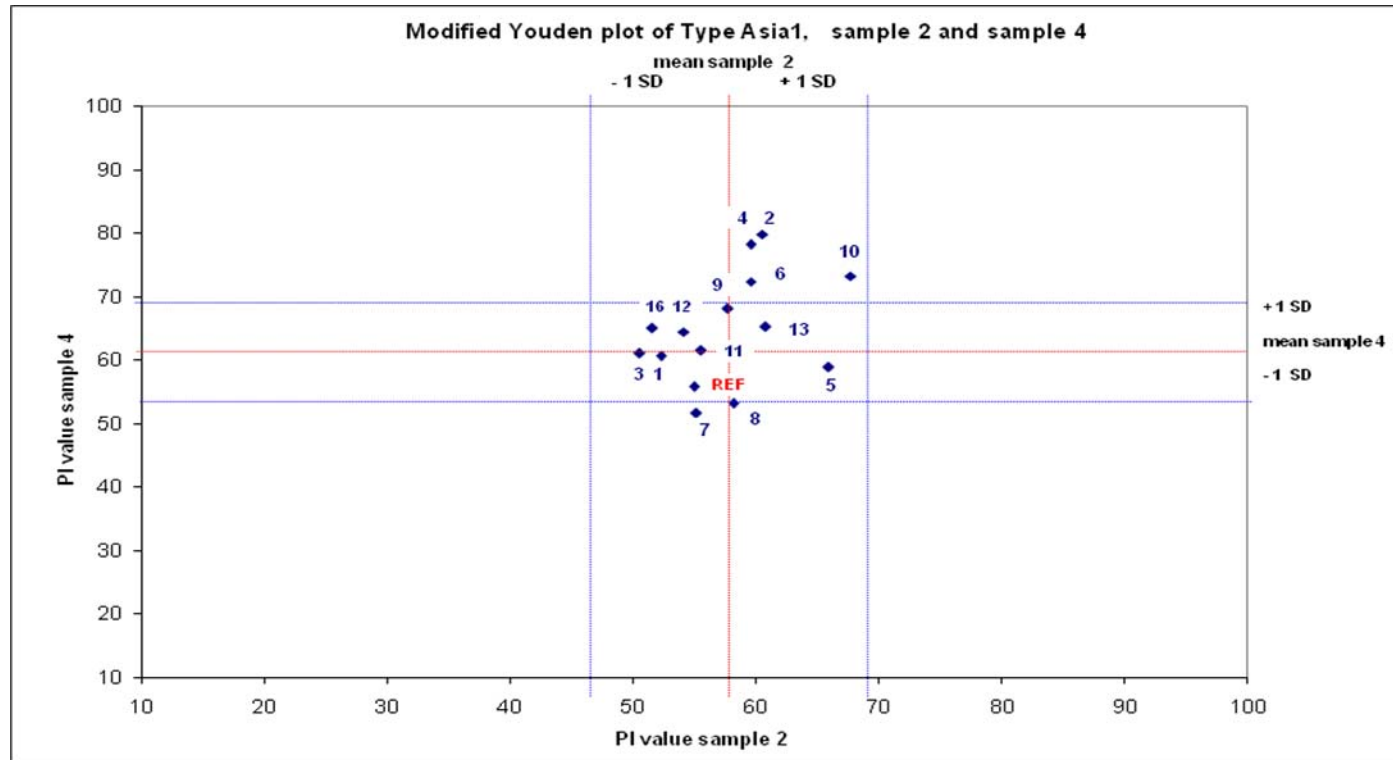
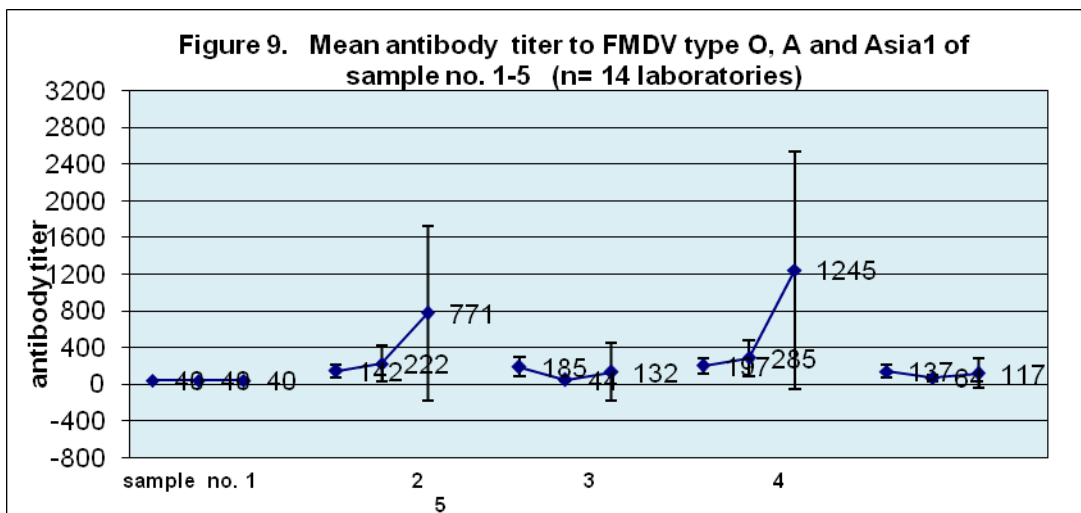


Figure 9. Result of mean antibody titer to FMDV type O, A and Asia1 in each laboratory and mean titer of reference data of sample no. 1- 5

Table 5. The result of NSP test and mean antibody titer from lab 1-Lab 16 compare with the REF. data

| Interlab serum | Type O | Type A | Type Asia | 3ABC | 3B |
|----------------|----------|-----------|------------|------|----|
| Sample 1 ± 1SD | <40 ±0 | <40 ±0 | <40 ±0 | - | ND |
| REF.data ± 1SD | <40±0 | <40±0 | <40±0 | - | - |
| Sample 2 ± 1SD | 143 ± 67 | 222±194 | 771 ±950 | - | ND |
| REF.data ± 1SD | 347±251 | 373 ±130 | 667 ±356 | - | - |
| Sample 3 ± 1SD | 15± 103 | 44 ±11 | 132 ±318 | + | ND |
| REF.data ± 1SD | 253±106 | <40 ±0 | 46 ±16 | + | + |
| Sample 4 ± 1SD | 197± 88 | 285 ± 197 | 1245± 1294 | - | ND |
| REF.data ± 1SD | 293 ±65 | 480 ± 175 | 1760 ± 934 | - | - |
| Sample 5 ± 1SD | 137 ±73 | 64 ± 41 | 117 ± 161 | - | ND |
| REF.data ± 1SD | 133 ±41 | 67 ±20 | 67 ±48 | - | - |



The NSP test results from all laboratories gave the similar results 5 by using the commercial kit, majority based on 3ABC NS reagent kit, (Priocheck), In case of NSP test using the commercial 3B NS kit (UBI) , just only Lab 1 was used either PrioCheck or UBI commercial kit and indicated the similar results by both tests. Sample no. 3 gave the result as NSP strong positive this resulting from the animal which was challenged with FMDV type O . In case of sample no. 2, 4 and 5 were the sera collected from vaccinated animal while sample no. 1 was negative serum from non vaccinated serum. However, some laboratories have not done the NSP test in this exercise therefore no result from these labs, such as Lab 10, Lab 11, Lab 12

(Detail and raw data of all tests by ELISA typing, LP ELISA and NSP test in each laboratory has been shown in the appendix)

6. Suggestions and observations from this interlaboratory comparison Programme round 1/12011

6.1 It is important that laboratories complete and return all requested forms and paperwork. Some laboratories have not returned the RRL with their test results, questionnaires and other document, due to some trouble in their assay procedure such as Lab 14, Lab 15 and Lab 17. These documents, questionnaires, test record forms, tracing sheets for reagent used, etc., therefore, it very useful for the evaluation or analysis of assay data might not sufficiently identify causes of errors and associated factors. However, in this report, the analysis of all information and data would be conducted from totally 14 laboratories to compare with the REFERENCE DATA of the provider.

6.2 One possible cause of test variability is the level of competency of laboratory personnel for testing and familiarity with the interlaboratory comparison process. For example, if there is poor technique in making serial dilutions, buffer preparation, checking pH of buffer, etc., variability in test OD results can occur.

6.3 In some laboratories it was evident that antigen controls and test samples in LP ELISA test had high ODs and were over the upper limit acceptance. This was most likely related to the technique for preparation of the antigen solution for each serotype. The reference antigen used in this test was concentrated and purified antigen that needed to be highly diluted before adding in the assay performance (the recommend working dilution was listed in the table 2).

6.4 In some laboratories a high OD occurred in test sample and the antigen control of the antigen typing test. This affected the test results and could have caused error in the final interpretation of results. It found that Lab 16, the interpretation of test sample was deviated from other laboratories such as a false negative result was produced in positive sample.

6.5 Plotting an internal quality control (IQC) chart of LP ELISA should be performed and reviewed regularly. The control chart should be checked (e.g. by reviewing control values from 40 plates over a 2 monthly interval) for undesirable trends. It is also recommended to have a verification test of all reference materials regularly by conducting antigen titration, rabbit trapping titration, guinea pig detecting titration, conjugate titration to ensure that all those reagents still giving an working dilution in acceptance limit. However the laboratories should have their good internal quality assurance control system in place and should evaluate their system regularly.

6.6 Some laboratories have less experience or understanding in adjusting the reagent working dilution. Adjustment of working dilution of control panel would require re-titration of reagents. In Lab 7, the working dilution of rabbit trapping, guinea pig detecting, and

control antigen of all 3 serotypes were adjusted without re-titration. This caused the test results of low antibody titer and deviate from other laboratories and reference data. Without re-titration, most of positive sera appeared to have a negative or very low antibody titer by LP ELISA.

7. Acknowledgements

The working group would like to thank Dr. Ronello Abila, OIE-SRR and OIE-RCU in supporting the 6th SEAFMD Laboratories Network Meeting, we would like to express our sincere thanks to Director General of Department of Livestock Development (DLD), Director of National Institute of Animal Health (NIAH), staff of Regional Research and Diagnostic Centers within Thailand and NIAH. We would like to thank staff of Regional Reference Laboratory for FMD (RRL) for their contribution and support in preparing materials, reagents and samples in this interlaboratory comparison programme round 1/2011 to meet the objectives and target goal, we would like to thank all head of SEAFMD National Laboratories in South East Asia region for their participating and the success of this project.

8. Reference

Axel Colling (1998). The External Quality Assurance Programme for use with the FAO/IAEA FMD LPB-Antibody ELISA, Animal production and Health Section. FAO/IAEA Agriculture and Biotechnology Laboratory, Seiberdoft, Austria.

9. Working group

Working group on sample and reagent preparation for using in this programme are listed follows;

- Dr. Wilai Linchongsubongkoch
- Dr. Dilok Aunpomma
- Dr. Kingkarn Boonsuya Seeyo
- Miss Piyaporn Chareonphon
- Miss Sopa Singkleebuthra
- Miss Janya Samanit
- Mrs. Ratanee Thingtha



(Wilai Linchongsubongkoch et al.)

Appendix

1. Raw data and OD value of ELISA typing test in each laboratory from Lab1 to Lab 16 including the Reference data.
(In this summary report, no data from lab 14, Lab 15 and Lab 17 were submission to the RRL provider)

2. Raw data and OD value of LP ELISA and NS test in each laboratory from Lab1 to Lab 16 including the Reference data
(In this summary report, no data from lab 14, Lab 15 and Lab 17 were submission to the RRL provider)