Support from the OIE Reference Laboratory for FMD to member countries

OIE Regional Workshop on Preparation and Submission of Dossiers for the Official Recognition of CSF and for the Endorsement of National Official Control Programme for FMD in Asia and the Pacific
Tokyo, 20-22 June 2017

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Department of Livestock Development, Thailand
1. Mission of OIE Reference Laboratory for FMD

2. Role of SEACFMD Laboratory Network

3. OIE Reference Laboratory network and SEACFMD Laboratory network for FMD

4. Training and technology transfer

5. FMD Research Collaboration

6. Guideline sample submission and evaluation of sample quality
1. Mission of OIE Reference Laboratory

1. To serve as the OIE Reference Laboratory for FMD diagnosis to member countries

2. To serve as the national laboratory for FMD within Thailand (total 8 Veterinary Diagnostic Centers and 1 NIAH, Bangkok)
3. Provide the FMD Diagnosis to member countries

:Standard diagnostic methods (Accredited by ISO 17025:2005);

1) Antigen detection; specimen is infected tissue, tongue epithelium, vesicular fluid or others

1.1 ELISA typing,

1.2 Virus isolation:
Pri. Lamb kidney cell
BHK21 cell line
ZZ-R 127

1.3 RT-PCR : (conventional or real time PCR)
2) Antibody detection; specimen is mainly serum

2.1 Liquid phase blocking ELISA (LP ELISA):
   To detect antibody titer to FMDV type O, A, Asia1 (structural protein)

2.2 NSP Test:
   To detect antibody to non-structural protein for differentiate infected from vaccinated animal (DIVA)
4. Strain characterisation

4.1 Vaccine matching and seed selection

**Objective:** To determine serological relationship between virus vaccine strain and field outbreak strain for seed vaccine selection

- Vaccine matching test
  - Complement Fixation (CF) Test
  - Virus Neutralization (VN) Test
  - Liquid phase blocking ELISA (LP ELISA)

**Interpretation**

\[ r = \frac{\text{Serum titer against heterologous field strain}}{\text{Serum titer against homologous vaccine strain}} \]

- \( r = 0.00-0.19 \) highly significant serological variation from the reference strain (poor matching)
- \( r = 0.20-0.39 \) significant difference from the reference strain, but protection may be satisfactory if using a sufficiently potent vaccine (moderate matching)
- \( r = 0.40-1.0 \) not significantly different from vaccine strain (good matching)
4.2 Genomic variation (sequencing):

Objective: To analyze genetic relationship among virus vaccine strain and virus field outbreak strains for tracing back to original virus causing outbreak in the field.
5. Production and providing of diagnostic reagents

- To the regional laboratories within Thailand
- To the SEACFMD laboratories in this region

6. Establishment Quality Assurance system in the region

* Organize the inter-laboratory comparison testing for SEACFMD laboratories and laboratories within Thailand
7. Training center and technology transfer in the region

8. Collaborative works among the institutes and international organizations: OIE, WRL, FAO, IAEA, QIA, AAHL & JICA
2. Role of SEACFMD Laboratory Network

- Rapid diagnosis of FMD viruses
  - Early detection and confirmation of FMD virus serotypes
  - Use of appropriate vaccine strain
- Facilitate sending of field isolates to Regional Reference Laboratory
- Strengthen surveillance
  - Molecular epidemiology
  - Mapping on the evolution of FMDV serotypes
- Capacity building on FMD diagnosis
  - Exchange visits of experts
  - Regular training programs
- Harmonization of diagnostic protocols
- Quality assurance system
3. FMD Laboratory network connection

- **National level**
  - RRL Thailand
    - VRDC BKK
    - VRDC Ratchaburi
    - VRDC Nakornrithi
    - VRDC Surin
    - VRDC Pisanulok
    - VRDC Chonburi
    - VRDC Lampooang

- **Regional level**
  - RRL Thailand
    - Vietnam
    - Philippines
    - Malaysia
    - Myanmar
    - Indonesia
    - Lao PDR

- **Global level**
  - 1st OIE REF Labnet meeting has established in 2006
  - 1st SEAFMD Labnet meeting has established in 2005 up to the present
1. Organize the training Course on FMD diagnosis; 9 regional FMD laboratories within Thailand were received knowledge technology transfer on Ag-typing and solving problem of collection and transportation of specimen.

2. Organize the Inter-laboratory comparison testing of FMDV serology test (LP ELISA, NSP and Ag-ELISA typing).

3. Providing ELISA reagents for FMD diagnosis to laboratories within Thailand.

4. Provide assistant and consultant on technical problem.
1. Providing the FMD diagnostic services, confirmation of diagnostic result and strain characterisation.

2. Providing ELISA reagents for FMD diagnosis to national laboratories in the SEA region.

3. Providing the inter-laboratory comparison or proficiency testing for member country in the region.

4. Participate in SEACFMD Laboratory Network meeting, the 1st lab network meeting has established in 2005 up to the present.

5. Strengthening in collecting field specimen and submission to RRL in good quality under the biosafety and biosecurity principle.
1. Participation in annual meeting of OIE/FAO Reference Laboratory network.

2. Participation on proficiency testing organized by WRLFMD, Pirbright Institute, UK.

3. Submission samples to WRLFMD, Pirbright annually for confirmation and further strain characterisation (vaccine matching and sequencing).

4. Participate in the 11th OIE/FAO Reference Laboratory network meeting was held in ANSES, France, 30 Nov-2 Dec 2016.

5. Participate in the Regional Meeting of OIE Reference Centres in Asia and the Pacific, 6-7 February 2017, Tokyo, Japan
2016 round: Inter-laboratory comparison or proficiency test organized by RRL

Inter-laboratory comparison round 4/2015-2016 organized by RRL FMD, Pakchong, , Thailand

Participating lab: 17 labs:
- 9 FMD laboratories within Thailand
- 8 SEAFMD laboratories, Cambodia, Lao PDR, Philippines, Malaysia, Myanmar
  Vietnam_ Hanoi, Vietnam_ Ho Chi Minh, Singapore, Thailand

1. ELISA Reagents providing:
   - Antigen detection and antibody detection for FMDV serotype O, A and Asia1
   - Rabbit trapping antibody for O, A and Asia1
   - Guinea pig detection antibody for O, A and Asia1
   - Control inactivated antigen for O, A and Asia1
   - Control serum for C++, C+ and C- for O, A and Asia1

2. Unknown samples:
   Unknown serum = 5 samples for LP ELISA and NSP test
   Unknown virus = 5 samples for antigen typing test

3. Additional document:
   Questionnaires, tracing sheet, record forms
   SOP if necessary for some lab

** 2017 round: Now is on going, 8 Labs within Thailand had already received, remained only SEA labs
EXAMPLE: ANALYSIS OF INTER-LAB RESULTS ROUND 4/2015-2016

**Figure 1** ELISA typing for serotype identification of FMDV type O, A and Asia1

**Figure 2** Overview of antigen typing result from each laboratory, Cut off OD > 0.20 defined as positive

**Figure 3** Overview RQC data of 10 antigens control C0 of FMDV type O, A and Asia1

**Figure 4** Overview RQC data of FMDV type O and Asia1 per serotype and laboratory

**Figure 5** Overview RQC data of FMDV type O, A and Asia1 per serotype and laboratory

**Figure 6** Overview RQC data of FMDV type O, A and Asia1 per serotype and laboratory

**Modified Youden plot**

**Figure 7** Type O: Plot Y value sample 1 and 4

**Figure 8** Type A: Plot Y value sample 2 and 3

**Figure 9** Type Asia1: Plot Y value sample 1 and 4

**Figure 10** Mean LP ELISA titer of serum sample no 1-5 and reference data of type O, A and Asia1

Result of NSP testing using commercial kits, 3ABC NSP and 3B NSP kit

<table>
<thead>
<tr>
<th>kit</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
<th>Sample 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>3ABC NSPs</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>3B NSPs</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Trouble shooting</td>
<td>Cause/factors</td>
<td>Recommendation</td>
<td></td>
<td></td>
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<td>---------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Control panel out of acceptance limit</td>
<td>Working dilution is not appropriate or wrong dilution</td>
<td>Re-titration of regents</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>2. High OD that occurred in test sample and the antigen control of the antigen</td>
<td>The technique for preparation of the antigen solution for each serotype.</td>
<td>Need more training and more experience for standardize all techniques</td>
<td></td>
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</tr>
<tr>
<td>typing</td>
<td>- Poor technique in making serial dilution, buffer preparation, checking pH</td>
<td>- Need checking the pH condition of all reagents</td>
<td></td>
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<tr>
<td></td>
<td>of buffer, etc.</td>
<td>- Check quality of purified water</td>
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<td></td>
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<tr>
<td>3. Irregular results or technician error</td>
<td>- Some laboratories do not set up the Internal Quality Control (IQC) in the</td>
<td>-Implement the IQC in test plate and regular monitoring</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>assay system</td>
<td>- Calibration of equipment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Essential equipment never done for calibration or verification</td>
<td>- Calibration of equipment</td>
<td></td>
<td></td>
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<tr>
<td>4. Less diagnostic knowledge in laboratory testing both on theoretically and</td>
<td>- Less experience in practicing and training regularly in scope</td>
<td>- Planning for annual training both within or out site the country</td>
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<tr>
<td>practically</td>
<td>of responsibility</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Changing of staff</td>
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</tbody>
</table>
4. Training and technology transfer in 2016

1. FMD antigen detection training course during 4-8 July 2016
   Participant from 9 Veterinary Research and Development Centers (VRDC) within Thailand and National Institute of Animal Health

2. Dr. Valerie Mioulet, Head of Virology Section, WRLFMD Institute, UK. visit RRL for investigation of testing Cambodia samples, 27 June –1st July 2016, OIE support.
3. AAHL, Australia: Dr. Singanallur, Nagendrakumar, Research collaborative work on vaccine matching. 29th July-11 August 2016

4. Dr. Sandar Lwin, Myanmar; Laboratory Training for FMD Diagnostics Capacity during 24th October 2016 - 13rd January 2017, under IAEA support

5. Trainee from Vietnam: training on FMD diagnosis and vaccine matching during 22th May to 2 June 2017
6. Research collaboration in 2016

1. NIAH-Japan and NIAH-Thailand; Training of RRL staff at Kodaira lab, Japan, on going research collaboration on complete genome sequencing in March 2016.

2. Animal and Plant Quarantine Agency (QIA), Korea and RRL, Thailand on exchange scientific visit and validation of developed rapid diagnostic kit for FMD serotype.
6. Research collaboration in 2017

3. Collaborative research in 2017;

- Training of RRL staff at Kodaira lab, Japan, on Immunohistochemistry testing

- Animal experiment with FMD Thai strain
  * on Virus shading and distribution of FMD A/Thai 46-1/2015 strain in experimentally infected cow and pig
  * This experimental result have been presented in the Thai-Japan Joint Conference at NIAH, Bangkok, June, 6-8 2017
7. Guideline for sample submission to laboratory in good quality

• To reduce problem effecting to diagnostic result

• To set up a standard and implement to the field veterinarians in collecting the good quality of samples/specimens for submitting to laboratory

• To introduce the use of biosafety and biosecurity principle to field veterinarians in packing and shipment of specimen to laboratory with safely
Packing of specimens following the biosafety and biosecurity principle
Evaluation of quality of FMD specimens submission to the RRL laboratory

- Summary of specimens collecting from field outbreak in Thailand and submission to laboratory during 2015-2016

- Type of specimen: tissue = 640
- Criteria for determine quality level, divided into 3 levels

  Good (3) = sample weight > 1.0 gm/volume > 1.0 ml
  Fair (2) = sample weight 0.4- 1.0 gm/volume 0.4- 1.0 ml
  Poor (1) = sample weight < 0.4 gm/volume <0.4 ml
### Table 1. Summary of diagnostic results compare with initial ELISA typing, virus isolation and RT-PCR (n= 640)

<table>
<thead>
<tr>
<th></th>
<th>Good (3)</th>
<th>Fair (2)</th>
<th>Poor (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial typing</td>
<td>Virus isolation</td>
<td>rRT-PCR</td>
<td>Initial</td>
</tr>
<tr>
<td>Positive</td>
<td>159/279 (60%)</td>
<td>259/279 (92.8%)</td>
<td>272/279 (97.5%)</td>
</tr>
<tr>
<td>Negative</td>
<td>120/200 (60%)</td>
<td>20/200 (10%)</td>
<td>7/7 (100%)</td>
</tr>
<tr>
<td>positive/ Total</td>
<td>159/279 (60%)</td>
<td>259/279 (92.8%)</td>
<td>272/279 (97.5%)</td>
</tr>
</tbody>
</table>

*Initial = ELISA typing using extraction of viral fluid
Virus isolation = passage of viral fluid onto cell culture, then confirm by ELISA typing*
Percentage of positive result in different sample quality (good, fair and poor)
Conclusion

• Quality of samples/specimens submission to laboratory
  Total sample = 640
  good (3) level = 43.6%
  fair (2) level = 35.3%
  poor (1) level = 21.1%

• Diagnostic results:
  * Initial samples;
    good (3) level = 60 % gave positive result
    fair (2) level = 23.5%,
    poor (1) level = 3.7 %

  * Isolated samples;
    good (3) level = 92.8 % gave positive result
    fair (2) level = 71.2%,
    poor (1) level = 48.9 %

• * rRT-PCR
  good (3) level = 97.5 % gave positive result
  fair (2) level = 88.9%,
  poor (1) level = 69.6 %
<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Total</th>
<th>VI / ELISA typing</th>
<th>RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>NVD Pos</td>
</tr>
<tr>
<td>Saliva*</td>
<td>44</td>
<td>17 (38.6%)</td>
<td>26 (59.1%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>18 (40.9%)</td>
</tr>
<tr>
<td>Oro-nasal* swap</td>
<td>44</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

*Saliva and oro-nasal swap samples were passaged onto primary lamp kidney cell, all samples could not be adapted

VI = Virus isolation
Guideline:
For packing and dispatch of sample or biological material via international airline following the biosafety and biosecurity principle

 Protocol sample submission to Regional Reference Laboratory for FMD in South East Asia via international airline

Prepared by
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Introduction
The Regional Reference Laboratory for Foot and Mouth Disease in South East Asia (RRL), National Institute of Animal Health, Department of Livestock Development (DLD), Ministry of Agriculture and Cooperatives in taking responsibility for FMD diagnosis and operating as the OIE Reference Laboratory for FMD and SEAFMD reference laboratory in the region. The specimens or infectious substances submitted to the laboratory for diagnostic purpose are received at DLD, Pakchong, Thailand by AIRFREIGHT ONLY. The destination airport is SUVARNABHUMI INTERNATIONAL AIRPORT. The customs clearance process will be preceded by the DLD officers and collected by the authorized staff from RRL, Pakchong.

In order to achieve the bio-safety and bio-security standard of samples or biological materials from overseas countries to Regional Reference Laboratory, the packing and dispatch of samples or biological materials are required as follows.
Example 1. Picture of IATA approved container for Infectious specimen

Example 2. Picture of IATA approved container for Non Infectious specimen
Example 3. An alternative method of packing with strong container is also acceptable for diagnostic reagents.
Recommendation:

1. Official document for Import permit /Export permit

2. Need knowledge and training on classification of infectious substance and packing of specimens under the IATA regulation.

3. Material safety data sheet (MSDS)
MATERIAL SAFETY DATA SHEET OF FMD SPECIMENS

(MSDS)

Responsible for specimen: Regional Reference Laboratory for FMD in South East Asia, Department of Livestock Development, Pakchong, Nakhonratchasima 30130, THAILAND
Tel: +66 44 279112, Fax: +66 44 314889

Description:

1. Specimen identification:
   - Liquid: samples fluid from FMD isolates virus
   - Use for in vitro research purpose only, use for identification of FMD serotype and sub-type and further investigation on strain characterization by nucleotide sequencing and determining of vaccine matching (r-value)

2. Information of specimen/samples:
   - The sample is the viral cultural fluid in BHK tissue culture cell using modified Eagle’s medium (MEM) as a culture medium.
   - The sample is sterilized by pass through 0.2 micron membrane filter and kept in sterilized cryovial with screw cap, volume of specimen contain 1 ml each. The vial cap is completely sealed with paraffin film in order to prevent leaking of specimen fluid to outside.

3. Risk identification:
   - The sample is classified as Risk Groups 3 Disease agents which is non hazardous to human but affecting animal only.

4. Packing specimen/sample:
   - The container is recommend to use the AAHL Infectious Materials Transport Container to meet the UN/ICAO/IATA regulation for transport of infectious materials.
   - The package has a UN Specification Marking (UN 602) indicating that the package is successfully tested designed:4H2/CLASS 6.2/02 AUS/356.CSIRO

5. Packaging specimen/sample:
   - Primary container, is metal can (small can), the sample fluid is placed into the small can, cotton wool is put as a cushion the sample within the primary container and to individually wrap and separate a number of smaller containers.
   - Before closing the lid, recommend to use an epoxy adhesive to seal around the lid.
   - Secondary container, is a metal can (big can) for insert the primary container into secondary container. Cover the top of primary container with the absorbent sheet, then put epoxy adhesive on the lid and press firmly to ensure full closure.
   - Outer packaging, is the moulded polystyrene box setting in the outer polypropylene box, put secondary container into place. It is recommended that the two freezer packs, pre-frozen at -20°C or dry-ice pellets to be inserted at either side of secondary container.
   - Enclose an itemized list of contents and placing on the polystyrene box accordance with IATA Packing Instruction 602 or 650, close the lid of outer box ensuring locking tag is in placed.

6. Overpack of container:
   - The IATA approved container of UN 602 or 650 can be overpacked by inserting in to the strong outer container which made of form and/or fiberboard box before shipping.
   - Dry ice is necessary to be used as a refrigerant to maintain freezing condition during transportation, it is recommended that the quantity of dry ice must be estimated to cover for the whole shipment.

7. Labelling and documentation labeling:
   - Using a waterproof pen or sticker write in the name and address and telephone of the person responsible for the shipment on the appropriate address label.
   - The content must be identified by the proper shipping name and number according with IATA classification eg. Infectious substance affecting animals UN2950, Diagnostic specimens or Biological products UN 3733
   - If dry ice is to be used as a refrigerant, a “Miscellaneous Goods” label must be attached to the top surface of the box.

8. Exposure control/ personal protection:
   - Sample fluid can be destroyed or inactivated by washing with alkali soap or any type of disinfectant such as 1% sodium hydroxide, 4% sodium carbonate, 0.1% glutaraldehyde solution or 0.25% iodine compound solution, then wash by tap water to clean up of disinfectant.
Thank you for your attention

Acknowledgements

RRL staff;
- Kingkarn Boonsuya Seeyo
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- Sopha Singkleebuth