

7th July 2020 SEACFMD National Coordinators (NC) Virtual Meeting

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Update on regional pilot testing of molecular diagnosis for FMD

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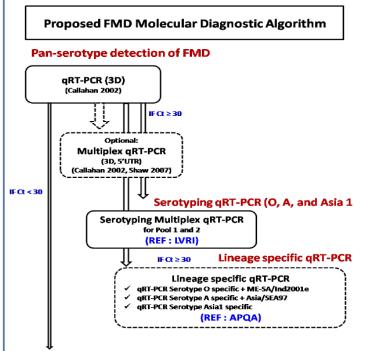
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Lanzhou Veterinary Research Institute

1st Regional Expert Group(REG) on FMD 14-16 May 2019, Bangkok





Sequencing of VP1 for genotyping

 Conventional RT-PCR using universal VP1 primers, and;
Direct sequencing of the PCR product (APQA SIK primers + Universal primers) Conclusions, Recommendations and Next steps

Session I: Ways to Improve Current Situation on FMD Molecular Diagnosis in the region

Conclusions:

- The Regional Expert Group (REG) agreed to draft a regional FMD molecular diagnostic algorithm for those countries in pool 1-2 that do not have a national diagnostic algorithm.
- The REG agreed that real-time RT-PCR methods are more sensitive than conventional RT-PCR methods, and thus are the preferred methods to be included in the initial steps of the regional diagnostic algorithm.
- 3. The REG agreed to use a 3-tiered approach towards molecular diagnosis for FMD, *in casu*:
 - Step 1: pan-serotype confirmatory detection of FMDV using real-time RT-PCR

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- Step 2: serotyping for O, A, Asia-1 using real-time RT-PCR
- Step 3: sequencing of the VP-1 region for genotyping
- 4. The experts drafted the following molecular diagnostic algorithm for FMD in Annex 1:
- 5. The REG agreed to share the draft algorithm to the experts that were unable to attend the first REG (*in casu* WRLFMD)



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Summary of tested results from 3 labs

| Serotyping RT-qPCR (O/A/Asia1)(LVRI) | | Lineage specific RT-qPCR (O/A/Asia1)(APQA) | | VP1 Sequencing with universal primers (Lee, 2012) | | VP1 sequencing with SIK primers (APQA) | |
|---|----------------------------|---|----------------------------|---|----------------------------|---|----------------------------|
| No of samples | Percentage of amplified(%) | No of samples | Percentage of amplified(%) | No of samples | Percentage of amplified(%) | No of samples | Percentage of amplified(%) |
| 64 | 90.63 | 64 | 81.25 | 54 | 38.89 | 54 | 77.78 |

Tested by 3 OIE Reference Laboratories

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- LVRI
- APQA
- RRL Pakchong



SEACFMD Laboratory Network Meeting

Pakchong, Thailand 4-5, November 2019

Recommendations

- 1. NOTE the progress of the members in the implementation of the previous SEACFMD LabNet recommendations, in particular in the use of RT-PCR;
- NOTE that FAO and OIE organized an FMD Regional Expert Group (REG) for Asia to provide technical recommendations to harmonize diagnostic procedures, in particular the molecular diagnostics and serological assays;
- AGREE to use the FMD molecular diagnostic algorithm recommended by the 1st Meeting of the FMD REG, and recommend Vietnam and Myanmar to test the algorithm at national FMD lab and provide feedback by February 2020;
- 4. ENCOURAGE members to use the protocol for sample collection recommended by FMD REG to improve the quality samples submission to laboratories;

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Regional pilot test for the FMDV molecular diagnostic algorithm TC March 13th, 2020

Study Design

1. Will test three steps molecular diagnostic scheme as recommended by REG using representative field isolates; including:

- •LVRI serotyping specific RT-qPCR protocols
- •APQA lineage(strain)-specific RT-qPCR protocols

•test the APQA primers and protocols for VP1 sequencing and test 2) the universal VP-1

amplification and sequencing protocol using representative field isolates at the same time;

- 2. The minimum number of samples for each test is <u>20-30 samples</u>
- 3. Results and raw data for each test will be reported using the template provided

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4. Malaysia, Myanmar and Vietnam will participate in



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Current arrangements

>APQA, Korea will supply lineage specific RT-qPCR kits (O, A and Asia1) to 3 countries

>LVRI, China will supply the primers and probes and the protocol for the serotyping O/A/Asia1 RT-qPCR to 3 countries.

➢RRL, Pakchong will supply the PCR controls (positive and negative) esp for FMDV O and A to a lab if needed.

OIE will supply Myanmar with master mix and extraction kits and some plastics.

>As soon as international flights resumes, APQA, LVRI and OIE will contact the 3 countries for the shipment.



Acknowledgement

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- ✓ Labs in Malaysia, Myanmar and Vietnam
- ✓ The WRL for FMD, The Pirbright Institute, UK
- ✓ OIE SEACFMD





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Thank you for your attention



