WORKSHOP ON FMD DIAGNOSTIC TECHNIQUES AND LABORATORY PRACTICES

APQA, SOUTH KOREA

2 – 13 December 2024

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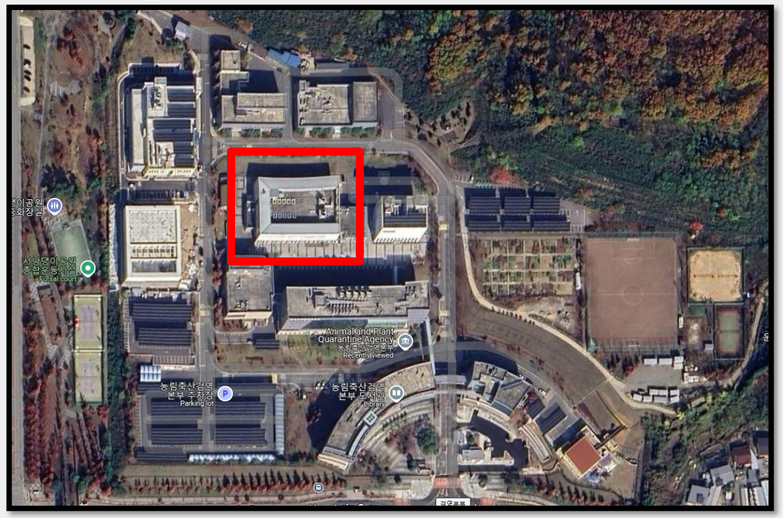
CONTENTS

INTRODUCTION

- Funded by IAEA, MAL5034-EVT2206735 SV on Reproductive System of Animal Disease, Tuberculosis and FMD
- No. of participant: one (1)
- Duration : 2 weeks (2 – 13 December 2024)
- Place: WOAH Reference Laboratory for FMD, Animal and Plant Quarantine Agency (APQA), South Korea



OBJECTIVES

- To learn the diagnostic techniques for FMD virus
- To discuss and learn about good laboratory practices in handling samples and conducting FMD testing
- To expand collaboration networks and facilitate knowledge exchange

COURSE MATERIALS

01 – SEROLOGY

02 – MOLECULAR

03 – TISSUE CULTURE

1. SEROLOGY

a. Non-Structural Protein (NSP) FMDV ELISA: Median Diagnostic kit & Bionote
b. Solid Phase Blocking ELISA (SPCE) serotype O : Median Diagnostic kit & Bionote

Median diagnostic kit (NSP)

Calculate the sample to negative ratio (S/N) by following the formula below:

$$SN = \frac{(Mean\ of\ sample\ OD)}{Mean\ of\ NC\ OD}$$

Result interpretation:

S/N value	Interpretation
S/N ≤ 0.60	Positive
S/N > 0.60	Negative

Bionote (NSP)

Calculation of the result (Percent Inhibition: PI)

- Calculate the mean value of the negative control (NCx/3)
- Calculate the mean value of the positive control (PCx/2)
- Calculation of PI as below;

$$PI\ value = (1 - \left(\frac{Average\ of\ OD\ sample}{Mean\ of\ NCX}\right)) x100$$

Interpretation of the test

Species	Cattle	Pigs	Goat
Positive	≥50 %	≥50 %	≥50 %
Negative	<50 %	<50 %	<50 %

Median diagnostic kit (SPCE)

Interpretation

- Calculate and determine the S/N value of the sample
- If the sample has an S/N value of 1.00 or higher, it is also indicated negative
- The formula for calculation is as below:

$$SN = \frac{(Mean\ of\ OD\ sample - Mean\ OD\ of\ positive\ control)}{(Mean\ of\ Negative\ control - Mean\ OD\ of\ Positive\ Control)}$$

S/N Value	≤ 0.60	> 0.60
Interpretation	Positive	Negative

Bionote (SPCE)

Result calculation

- Calculate the average OD of the negative control solution (NCx).
- Used the Percentage Inhibition (PI) value as formula below;

$$PI\ value = (1 - \frac{average\ OD\ of\ sample}{NCx})) \ x\ 100$$

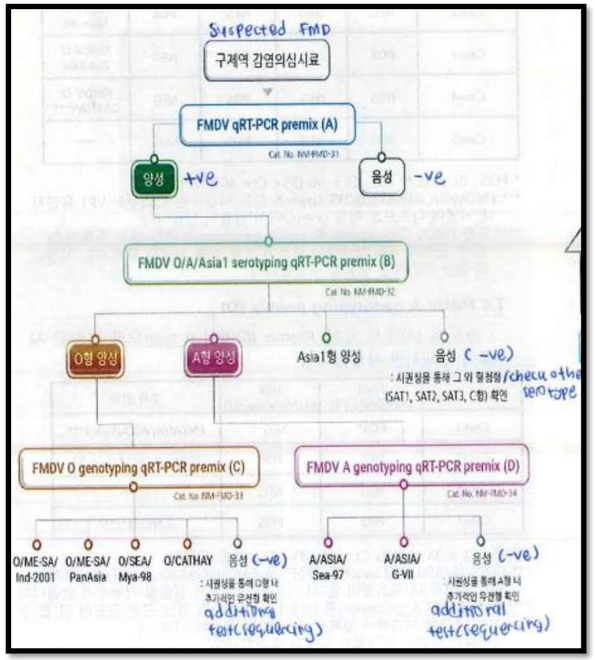
Interpretation

PI value of sample	Interpretation
PI value <50	Negative
PI value >50	Positive

2. MOLECULAR

FMDV Multiplex Real-time PCR kit: Powercheck

- ❑ Ready to use kit
- ❑ To detect FMDV using 2 assay: 3D and IRES
- ❑ Fluorescent dye: FAM (3D), JOE (IRES) & Cy5 (IC)



FMDV Multiplex for Serotyping qRT-PCR: Median

- ❑ Premix: FMDV, O/A/Asia1 serotyping, O genotyping & A genotyping
- ❑ Premix FMDV: to detect FMD virus
- ❑ Premix O/A/Asia1 serotyping: to detect serotype
- ❑ Premix O genotyping: to detect lineage – PanAsia, Ind2001, CATHAY or Mya-98
- ❑ Premix A genotyping: to detect lineage – A/ASIA/G-VII or Sea-97

2. MOLECULAR

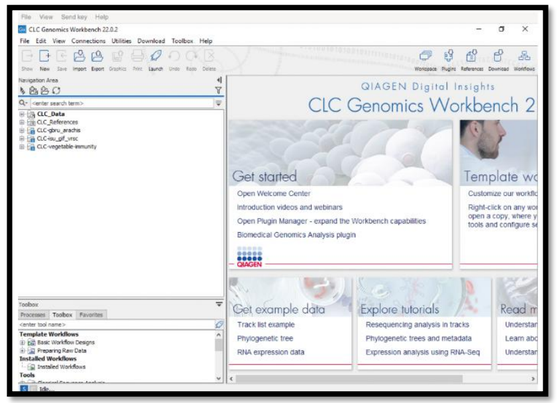
Sequencing of FMD virus

- ❑Conventional sequencing (1st generation)
  - Sanger's method : Seq studio (ABI), VP1
- ❑NGS (2nd generation)
  - Sequencing by synthesis : iSeq (illumina) , WGS
- ❑NGS (3rd generation)
  - Real time sequencing: Minlon (ONT), WGS

iSeq (Illumina)

Cartridge (Illumina)

Sample preparation → Library preparation → Library sequencing → Data analysis



QIAGEN CLC Genomics Workbench (data analysis)

Minlon Flow cell

Minlon

2. MOLECULAR

FMDV multiplex Real-time RT-PCR using POCT

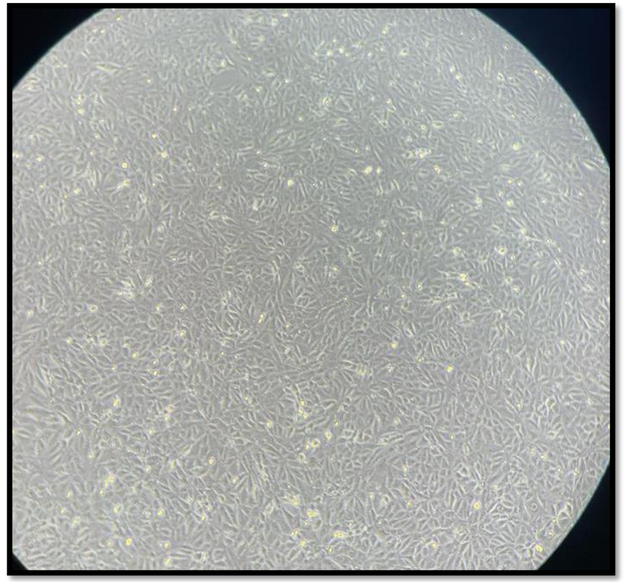
- ❑ Using cartridge & LOAA^{M} analyser
- ❑ One cartridge have 9 targets: FMDV (3D), serotype (O,A,Asia1), positive control, SVDV & SVV

3. TISSUE CULTURE

❑Subculture of cell lines:

- Baby Hamster Kidney (BHK-21)
- Porcine kidney cell line (IBRS-2)
- Fetal porcine kidney cell line (LFBKαvβ6 or MGPKαvβ6)

BHK-21



IBRS-2

LFBKαvβ6

SUGGESTIONS

Collaboration with international experts

- Propose the establishment or strengthening partnerships with international reference laboratories to share knowledge on practices, research, and diagnostics.

Become member of Global FMD Research Alliance (GFRA)

- To stay updated on research, diagnosis, and control strategies

Improve laboratory capabilities

- Increase budget for high technology equipment and software

THANK YOU