VDRG® FMDV 3Diff/PAN Ag Rapid kit
Cat. No. PM-FMD-16
For in-vitro veterinary diagnostic use only

General Description
VDRG® FMDV 3Diff/PAN Ag Rapid kit is a lateral flow chromatographic immunoassay for universal detection of all seven serotypes (PAN) of Foot-and-mouth disease virus (FMDV) antigen and simultaneous typing of O, A and Asia1 serotypes (3Diff) in saliva, tissue or vesicular fluid around lip, tongue, gums, nose, hoof of porcine or bovine.

PAN strip of the device detecting all seven FMDV serotypes using a pan-serotype detection antibody conjugate and capture antibody line determines the existence of detectable level of FMDV in specimens diluted in Sample Dilution Buffer.

3Diff strip of the device specifically determines FMDV O, A and Asia1 serotype antigens in specimens diluted in sample dilution buffer using a pan-serotype detection antibody conjugate and three serotype-specific capture antibody lines.

FMDV antigen bound to antibody-gold particle conjugate will flow through nitrocellulose membrane by capillary action to be captured at test line coated with pan-serotype or serotype O, A and Asia1 specific antibodies. The accumulation of gold particle on the test line will form a red colored line. The test procedures can be completed at 15 minutes after application of specimens.

Device Appearance
Specimen application holes (S1 and S2) are located at right side of test device; diluted specimens will be applied to these positions. The test (T) and control (C) lines in rectangular open display are marked on the both sides of the device. The sample pad, conjugate pad, nitrocellulose membrane, and absorption pad are assembled inside cassette of the test device.

WOAH Statement
The validation data for this kit have been certified in May 2023 by WOAH, based on expert review, as fit for the following purposes:

VDRG® FMDV 3Diff/PAN Ag Rapid kit is a lateral flow test or pen-side test intended for the universal detection of foot-and-mouth disease virus (FMDV) of serotypes A, O and Asia-1 in tissue samples (epithelium) or fluid from blisters or ruptured lesions of suspected swine or cattle. The test is designed to be used for the rapid diagnosis of foot-and-mouth disease virus infection in samples from swine or cattle.

Sample Preparation

1. Specimen collection
A. Collection of fluid from intact vesicle: Draw vesicular fluid using a syringe.
B. Collection of fluid from ruptured vesicles: Soak vesicular fluid using a cotton swab.
C. Tissue sampling from ruptured lesions.
   ① Follow the instruction manual in VDRG® Tissue Sample Extraction kit (CAT. NO. EXT-TIS-11, not provided).
   ② Collect 0.2g of fresh and friable epithelium (size of little finger nail) from surface or margin of vesicles or other tissues of interest.
D. Saliva: Collect saliva from porcine or bovine using appropriate method.
   ① For bovine, collect saliva directly from tongue using disposable plastic gloves.
   ② For swine, collect saliva using chewing rope or other oral fluid collection kit.
E. Cultured virus: Collect virus culture media using micropipette.

2. Sample processing
A. Syringe-collected fresh vesicular fluid
   ① Add 1 scale (approximately 250ul) of Sample Dilution Buffer to the Test Tube using dropper.
   ② Add 250ul of syringe-collected vesicular fluid to the Test Tube and mix gently.
B. Swab-collected fluid from ruptured vesicles
   ① Add 2 scales (approximately 500ul) of Sample Dilution Buffer to the Test Tube using dropper.
   ② Soak the swab in the dilution buffer, mix by swirling and extract the vesicular lesion fluid by pressing the cotton bud against the tube wall.
   ③ Remove the swab from the Test Tube after extraction.
C. Tissue-extracted fluid
   ① Follow the instruction manual in VDRG® Tissue Sample Extraction kit (CAT. NO. EXT-TIS-11, not provided).
   ② Add 4 scales (1ml) of Sample Dilution Buffer to the extraction vial.
   ③ Add tissue sample to the extraction vial.
   ④ Cut the tissue into pieces using scissors and grind using pestle and sand included in the kit.

Kit Components

<table>
<thead>
<tr>
<th>No.</th>
<th>Reagents</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FMDV 3Diff/PAN Ag Rapid Test Device</td>
<td>10 tests</td>
</tr>
<tr>
<td>2</td>
<td>Sample Dilution Buffer</td>
<td>1 bottle</td>
</tr>
<tr>
<td>3</td>
<td>Test Tube</td>
<td>10 ea</td>
</tr>
<tr>
<td>4</td>
<td>Swabs</td>
<td>10 ea</td>
</tr>
<tr>
<td>5</td>
<td>Dropper</td>
<td>10 ea</td>
</tr>
<tr>
<td>6</td>
<td>Instruction Manual</td>
<td>1 copy</td>
</tr>
</tbody>
</table>

Validated and certified by WOAH as fit for the purposes defined in this instruction manual.
Registration number: WOAH 022029

MEDIAN Diagnostics Inc.

- 1 -
D. Saliva
1. Add 2 scales (approximately 500ul) of Sample Dilution Buffer to the Test Tube using the dropper.
2. Centrifugate(6,000rpm, 10min) the collected saliva and soak the swab with supernatant of centrifugated saliva.
3. Soak the swab in the dilution buffer, mix by swirling and extract the saliva by pressing the cotton bud against the tube wall.
4. Remove the swab from the Test Tube after extraction.
5. Use the clarified fluid for testing.

E. Virus culture media
1. Add 200ul of Sample Dilution Buffer to the Test Tube, Eppendorf tube or microplates.
2. Add 200ul of virus culture media to the Test Tube and mix by several times of swirling.

**SUMMARY OF VALIDATION STUDIES**

### Analytical specificity

**Conclusion:** The kit did not respond to other viruses causing vesicular lesions like the clinical symptoms of FMDV, namely Vesicular stomatitis virus, Swine vesicular disease virus and Seneca valley virus. In addition, there was no cross-reaction for other serotypes in each line.

<table>
<thead>
<tr>
<th>No.</th>
<th>Virus name</th>
<th>Cross-reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vesicular stomatitis virus</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>Swine vesicular disease virus</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>Seneca Valley virus</td>
<td>No</td>
</tr>
</tbody>
</table>

### Analytical sensitivity

**Conclusion:** The detection limit was measured by serially diluting the virus culture solution 10-fold using the negative samples. The virus culture solution was previously titrated with TCID<sub>50</sub>/ml. And it was compared with Ag ELISA (FMDV ANTIGEN DETECTION and SEROTYPING ELISA (FMDV O, A, C, Asia1, SAT1-2, Pirbright, UK)) and RT-PCR (Accupower FMDV Real-Time RT-PCR MasterMix kit, BIONEER). Although there is a slight difference from strain to strain, this Ag Rapid kit could detect up to 1.12x10<sup>10</sup>TCID<sub>50</sub>/ml for type O, up to 1.21x10<sup>10</sup>TCID<sub>50</sub>/ml for type A, and 8.43x10<sup>5</sup>TCID<sub>50</sub>/ml for type Asia1. The limit of detection (LoD) of this Ag rapid kit for Type SAT1 was 8.43x10<sup>4</sup>TCID<sub>50</sub>/ml, Type SAT2 was 7.9x10<sup>4</sup>TCID<sub>50</sub>/ml, Type SAT3 was detectable up to 5.01x10<sup>4</sup>TCID<sub>50</sub>/ml Type Asia1 was 3.2x10<sup>5</sup>TCID<sub>50</sub>/ml, Type SAT1 was 2x10<sup>5</sup>TCID<sub>50</sub>/ml, Type SAT2 was 9.4x10<sup>4</sup>TCID<sub>50</sub>/ml, Type SAT3 was detectable up to 5.01x10<sup>4</sup>TCID<sub>50</sub>/ml when using the spiked viral culture solution in 20% homogenate of tissue.

### Limit of detection (Saliva spiking)

#### Sensitivity

- **FMDV-positive samples in Korea, Vietnam, Myanmar**: Sensitivity in bovine: 98.35% (n=595/605), (95% CI: 96.98% ~ 99.20%)
- **Sensitivity in swine**: 99.1% (n= 544/549), (95% CI: 97.89% to 99.70%)

#### Specificity

- **FMDV-negative Saliva in Korea (RT-PCR)**: Specificity in bovine: 100% (n=92/92), (95% CI: 96.07% to 100.00%)
- **Specificity in swine**: 99.5% (n= 398/400), (95% CI: 98.21% to 99.94%)

#### Diagnostic characteristics:

- **Threshold determination and Diagnostic sensitivity (DS<sub>e</sub>) and specificity (DS<sub>f</sub>) estimates**

**Conclusion:** Sensitivity, specificity, and CI values were calculated by using https://www.medcalc.org/calc/diagnostic_test.php

1. **Sensitivity**
   - **FMDV-positive samples in Korea, Vietnam, Myanmar**
   - Sensitivity in bovine: 98.35% (n=595/605), (95% CI: 96.98% ~ 99.20%)
   - Sensitivity in swine: 99.1% (n= 544/549), (95% CI: 97.89% to 99.70%)

2. **Specificity**
   - **FMDV-negative Saliva in Korea (RT-PCR)**
   - Specificity in bovine: 100% (n=92/92), (95% CI: 96.07% to 100.00%)
   - Specificity in swine: 99.5% (n= 398/400), (95% CI: 98.21% to 99.94%)

#### Reproducibility

**Conclusion:** Using a series of three-lot products, three independent operators tested the standard substance (O, A, Asia1 each strong, medium, weak positive sample, negative 4 samples, total 13 samples) twice a day for 10 days per lot. The within-run, between-run, between-day and within-laboratory precision test results were all determined to be consistent. Three experimenters tested reproducibility with three lots of products and found that 100% of the results were consistent.

- **Diagnostic reproducibility**
  - **Using a series of products, researchers in two different diagnostic laboratories** tested the standard substance (O, A, each 2 strong, medium, weak positive samples, negative 4 samples, total 16 samples) twice a day for 3 days per lot. There 2 different results in weak positive samples and all the other determined to be consistent.

**Conclusion:** Two different labs tested reproducibility, and 99.5% of the results were consistent.
TEST PROCEDURE

1. Remove a VDRG® FMDV 3Diff/PAN Rapid Test Device from the foil pouch and place on a flat surface.
2. Slowly add 4 drops (100ul) of the processed sample solution to position “S1” and “S2” on the test device using a Dropper (provided) or micropipette (not provided).
3. Read results at 15 minutes exactly. Reading later than 15 minutes may cause inaccurate results.

PRECAUTIONS

1. For in-vitro animal diagnostic use only.
2. Read this instruction manual thoroughly and follow all steps strictly for successful use of the product.
3. Extended exposure of this Rapid Test Device to moisture may decrease test performance. Therefore, open the device right before use (<10 minutes).
4. Make sure to use a separate Test Tube, Dropper, and Swab for each sample.
5. Do not touch the membrane in the device. The results may be affected if touched.
6. Do not use test device and reagents after expiration date.
7. Wear personal protective equipment (PPE) such as lab coat, goggle, and disposable gloves while performing the assay. Wash hands thoroughly afterwards.
8. All test samples should be considered potentially infectious and all items contacting the samples should be considered contaminated.
9. After use, all wastes should be sterilized with high-pressure steam at 121 degrees Celsius for ≥15 minutes or comparable methods.
10. This Rapid Kit is considered as a preliminary test. The result should be confirmed by other laboratory tests for confirmatory diagnosis.
11. Sometimes, if the sample has low titer of O, A or Asia1 FMDV, the result may be shown as positive in strip PAN and negative in strip 3Diff. In this case, additional tests should be done.

STORAGE AND STABILITY

Store all reagents at 2~30°C. Do not freeze. Reagents remain stable through the expiration date when stored as instructed.

TEST METHOD SUMMARY

Specimen collection & processing

Device preparation

Add processed sample solution (4 Drops)

Reading the results

REFERENCE


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