Rapid Foot-and-Mouth Disease Virus Antigen Test Kit

Document Number: SKR-IFU-VFG70227
Revised Date: 14 February 2023

For Veterinary Use Only

READ ALL INSTRUCTIONS BEFORE BEGINNING THE TEST

RIDX™ FMDV 3Diff/PAN Ag Combo Test Kit

[CAT No. LGM-VFG-71]

Introduction

Foot-and-mouth disease is an acute, systemic disease of domestic and wild cloven-hooved animal species and is caused by Foot-and-mouth disease virus (FMDV), the prototype member of the genus *Aphthovirus* of the family Picornaviridae[1]. FMDV occurs as seven distinct serotypes (Euroasiatic serotypes A, O, C, and Asia1 and South African Territories (SAT) serotypes SAT1, SAT2, and SAT3) and multiple subtypes reflecting significant genetic variability[1].
The disease is characterized by fever and vesicles in the mouth and on the muzzle, teats, and feet and is spread through direct contact or aerosolized virus via respiratory secretions, milk, semen, and ingestion of feed from infected animals[2]. In a susceptible population, morbidities reach 100% and mortalities are rare except in young animals[3, 4].

Principle

The RIDX™ FMDV 3Diff/PAN Ag Combo Test Kit is a lateral flow chromatographic immunoassay for the qualitative detections of all seven serotypes antigens of FMDV in bovine or porcine samples.

• 3Diff Ag Test: FMDV 3Diff strip detects FMDV Asia1, type A, and type O antigens. Three test lines (AS, A, and O) and the control (C) line on the surface of the device.
• PAN Ag Test: FMDV PAN strip detects all seven (7) serotypes (type O, type A, type C, Asia1, SAT1, SAT2, and SAT3) of FMDV antigens. The test line (PAN) and the control (C) line on the surface of the device.

If the FMDV antigens exist in the sample, that bind to the gold-conjugated FMDV antibodies. The complexes move through the membrane by capillary force and respond to the FMDV antibodies on the test line, resulting in a red line. The control line indicates that the test is performed correctly. The highly selective and sensitive four monoclonal antibodies to FMDV are used as capture and detector in the RIDX™ FMDV 3Diff/PAN Ag Combo Test Kit. These antibodies are capable of detecting FMDV antigens in bovine and porcine samples with high accuracy.

Performances

1. Clinical Sensitivity & Clinical Specificity

1) 3Diff strip: FMDV serotype O Ag

		Disease Status		
		+	-	Total
RIDX™ FMDV 3Diff O line	+	60	2	62
	-	8	490	498
	Total	68	492	560

Clinical Sensitivity: 88.24% (60/68, 95% CI*: 78.47% ~ 93.92%)
Clinical Specificity: 99.59% (490/492, 95% CI: 98.53% ~ 99.89%)
Diagnostic Accuracy: 98.21% (550/560, 95% CI: 96.74% ~ 99.03%)

* CI: Confidence Interval

2) 3Diff strip: FMDV serotype A Ag

		Disease Status		
		+	-	Total
RIDX™ FMDV 3Diff A line	+	70	2	72
	-	0	490	490
	Total	70	492	562

Clinical Sensitivity: 100% (70/70, 95% CI: 94.80% ~ 100%)
Clinical Specificity: 99.59% (490/492, 95% CI: 98.53% ~ 99.89%)
Diagnostic Accuracy: 99.64% (560/562, 95% CI: 98.71% ~ 99.90%)

3) 3Diff strip: FMDV serotype Asia1 Ag

		Disease Status		
		+	-	Total
RIDX™ FMDV 3Diff AS line	+	60	2	62
	-	0	490	490
	Total	60	492	552

Clinical Sensitivity: 100% (60/60, 95% CI: 93.98% ~ 100%)
Clinical Specificity: 99.59% (490/492, 95% CI: 98.53% ~ 99.89%)
Diagnostic Accuracy: 99.64% (550/552, 95% CI: 98.69% ~ 99.90%)

4) PAN strip: FMDV serotype O Ag

		Disease Status		
		+	-	Total
RIDX™ FMDV PAN T line	+	68	2	70
	-	0	490	490
	Total	68	492	560

Clinical Sensitivity: 100% (68/68, 95% CI: 94.65% ~ 100%)
Clinical Specificity: 99.59% (490/492, 95% CI: 98.53% ~ 99.89%)
Diagnostic Accuracy: 99.64% (558/560, 95% CI: 98.71% ~ 99.90%)

5) PAN strip: FMDV serotype A Ag

		Disease Status		
		+	-	Total
RIDX™ FMDV PAN T line	+	70	2	72
	-	0	490	490
	Total	70	492	562

Clinical Sensitivity: 100% (70/70, 95% CI: 94.80% ~ 100%)
Clinical Specificity: 99.59% (490/492, 95% CI: 98.53% ~ 99.89%)
Diagnostic Accuracy: 99.64% (560/562, 95% CI: 98.71% ~ 99.90%)

6) PAN strip: FMDV serotype Asia1 Ag

		Disease Status		
		+	-	Total
RIDX™ FMDV PAN T line	+	60	2	62
	-	0	490	490
	Total	60	492	552

Clinical Sensitivity: 100% (60/60, 95% CI: 93.98% ~ 100%)
Clinical Specificity: 99.59% (490/492, 95% CI: 98.53% ~ 99.89%)
Diagnostic Accuracy: 99.64% (550/552, 95% CI: 98.69% ~ 99.90%)

2. Limit of Detection (LOD)

Serotype	LOD ($TCID_{50}$/mL)	
	3Diff strip	PAN strip
O	0.56×10^4	0.56×10^4
A	1.00×10^4	0.56×10^4
C	-	0.56×10^4
Asia1	4.22×10^4	4.22×10^4
SAT1	-	4.22×10^5
SAT2	-	0.75×10^5
SAT3	-	3.69×10^4

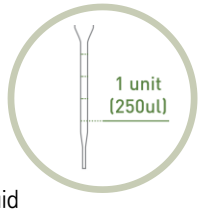
3. There is no cross-reactivity with classical swine fever virus, porcine reproductive and respiratory syndrome virus, seneca valley virus, swine vesicular disease virus, and vesicular stomatitis virus.
4. Differential diagnosis of 3 different serotypes (O, A, and Asia1)
5. Concurrent diagnosis both of a viral common antigen (all 7 serotypes) and serotype-specific antigens (O, A, and Asia1) of FMDV

Kit Components

	Component	Number/Kit
1	FMDV 3Diff/PAN Ag combo test device	10
2	Sample dilution buffer	1
3	Disposable swab	10
4	Disposable dropper	10
5	Sample tube	10
6	Instructions for use	1

Storage & Stability

1. Store the test kit at 2~30℃ (35.6~86℉). Do not freeze.
2. Do not store the test kit in direct sunlight.
3. The test kit is stable within the expiration date marked on the package label.



Sample Preparation

[Vesicular fluid]

1. Collect the vesicular fluid from a blister with a syringe. If the blister bursts, collect the vascular fluid with a disposable swab.
2. Sample from intact blister

1) Transfer 1 unit (approximately 250μL) of the sample dilution buffer to the sample tube using dropper.
2) Add 250μL of vesicular fluid to the sample tube and mix gently with the sample dilution buffer.

3. Sample from burst blister

1) Transfer 2 units (approximately 500μL) of the sample dilution buffer to the sample tube using dropper.
2) Put sample-soaked swab into the dilution buffer and swirling gently.
3) Press the cotton swab against the wall of the tube to extract the sample.
4) Remove the swab from the test tube after extraction.

[Saliva]

1. Sample collection
• Bovine: Collect saliva from bovine's tongue directly.
• Porcine: Use a chewing rope or other oral fluid collect kits.
2. Use the impurities free saliva sample. Centrifuge the saliva samples (6,000rpm, 10min), if the samples have impurities (The separate container for centrifugation is not provided in this kit).
3. Transfer 2 units (approximately 500μL) of sample dilution buffer to the sample tube using a dropper.
4. Soak a swab with supernatant of the centrifuged saliva.
5. Put sample-soaked swab into the dilution buffer and swirl gently.
6. Press the cotton swab against the wall of the tube to extract the sample.
7. Remove the swab from the test tube after extraction.

[Cultured virus]

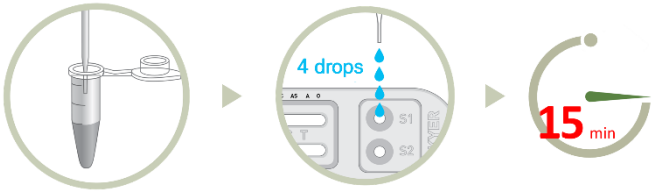
1. Transfer 200μL of the sample dilution buffer to the sample tube, Eppendorf tube or microplates.
2. Collect and add 200μL of virus-cultured media by using the micropipette to the sample tube and swirl the tube several times to mix.

[Sample storage]

The samples should be tested immediately after collection. If samples cannot be tested immediately, they should be stored at −20°C (−4℉). After mixing with the sample dilution buffer, do not store under any conditions, and do not use this mixed & stored sample for testing.

Test Procedure

1. All reagents and samples must be at room temperature (15~30℃ /59~86℉) before use.
2. Take the supernatant of the prepared sample solution by using a dropper.
3. Add 4 drops (100μL) of the sample solution into each sample hole on the test device slowly and vertically.
4. Read test results at 15 minutes.



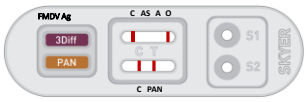
[Summary of Test Procedure]

Interpretation of Results

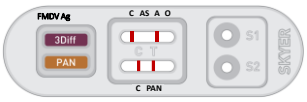
1. Positive results

Test (T) line and control (C) line within the result window indicate the presence of FMDV antigens.

1) Serotype O positive

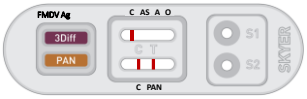


2) Serotype A positive



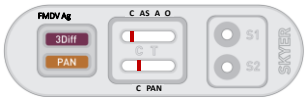
3) Serotype Asia1 positive

4) Serotype C, SAT1, 2, 3 positives



2. Negative result

Only control (C) line appears in the result window.



3. Invalid results

If the control (C) line does not appear, the result might be considered invalid. The sample should be retested.

Precautions

1. This test kit is for veterinary *in vitro* diagnosis only especially bovine and swine. Do not use this test kit for other animals.
2. The test device is sensitive to humidity and heat. Use the test device within 10 minutes after removing the foil pouch.
3. Do not touch the membrane of the test device.
4. Do not use the test device if the foil pouch is damaged.
5. Do not use an expired test kit.
6. Do not reuse the test components (device, dropper, swab, and sample tube).
7. Do not mix components from different lot numbers because the components in this kit have been QC tested as a standard batch unit.
8. Decontaminate and dispose of all samples, used kits, and potentially contaminated materials following national and local regulations.
9. All samples should be handled as being potentially infectious. Wear protective gloves while handling samples. Wash hands thoroughly afterward.

References

1. Carrillo C, Tulman ER, Delhon G, Lu Z, Carreno A, Vagnozzi A, Kutish GF, Rock DL. Comparative genomics of foot-and-mouth disease virus. *J Virol*. 2005; 79(10): 6487-6504.
2. Arzt J, Baxt B, Grubman MJ, Jackson T, Juleff N, Rhyan J, Waters R, Rodriguez LL. The pathogenesis of foot-and-mouth disease II: viral pathways in swine, small ruminants, and wildlife; myotropism, chronic syndromes, and molecular virus-host interactions. *Transbound Emerg Dis*. 2011; 58(4): 305-326.
3. Kitching RP. Clinical variation in foot and mouth disease: cattle. *Rev Sci Tech Off Int Epiz*. 2002; 21(3): 499-504.
4. Kitching RP, Alexandersen S. Clinical variation in foot and mouth disease: pigs. *Rev Sci Tech Off Int Epiz*. 2002; 21(3): 513-518.

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