World Organisation for Animal Health
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WOAH Procedure for Registration of Diagnostic Kits
Validation Studies Abstract

Name of the diagnostic kit: VDRG® FMDV 3Diff/PAN Ag Rapid kit

Manufacturer: MEDIAN Diagnostics Inc.

Procedure /Approval number: WOAH 022029

Date of Registration: May 2023

Disease: Foot and Mouth Disease (FMD) in swine and cattle.

Pathogen Agent: Foot and Mouth Disease virus (FMDV)

Type of Assay: Lateral flow test or pen-side test

Purpose of Assay: The 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) and differentiation 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.

Species and Specimens

Tissue samples (epithelium) or fluid from blisters or ruptured lesions of suspected swine or cattle

1. **Information on the kit**
   Please refer to the kit insert available on the WOAH Registry web page or contact the manufacturer at MEDIAN Diagnostics Inc.
2. **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.

No.	Virus name	Cross-reaction
1	Vesicular stomatitis virus	No
2	Swine vesicular disease virus	No
3	Seneca Valley virus	No

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 $TCID_{50}$/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 (AccupPower 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 $1.12x10^4TCID_{50}$/ml for type O, up to $1.12x10^4TCID_{50}$/ml for type A, and $8.43x10^4TCID_{50}$/ml for type Asia1. The limit of detection (LoD) of this Ag rapid kit

for Type SAT1 was 8.43x10^{5}TCID$_{50}$/ml; Type SAT2 was 1.5x10^{5}TCID$_{50}$/ml, and Type SAT3 was up to 7.38x10^{4}TCID$_{50}$/ml when using the spiked viral culture solution in saliva.

Type O was detectable by 5.01x10^{4}TCID$_{50}$/ml, Type A was 3.16x10^{4}TCID$_{50}$/ml, Type Asia1 was 3.2x10^{4}TCID$_{50}$/ml, Type SAT1 was 2x10^{5}TCID$_{50}$/ml, Type SAT2 was 7.9x10^{4}TCID$_{50}$/ml, Type SAT3 was detectable up to 5.01x10^{4}TCID$_{50}$/ml when using the spiked viral culture solution in 20% homogenate of tissue.

Limit of detection (Saliva spiking)

Serotype	Strain	Topotype	TCID$_{50}$/□	Rapid Cut-off		
				VDRG FMDV 3Difff/PAN(TCID$_{50}$/ml)		RT-PCT
				Strip 3Diff	Strip PAN	Ct value
O	Jin-cheon	SEA/Mya-98	1.5x10^6	1.5x10^4	1.5x10^4	24.94
O	O/Hapcheon/KOR/2014	SEA/Mya-98	1.12x10^6	1.12x10^4	1.12x10^4	26.91
O	Gim-je	SEA/Mya-98	1.5x10^6	1.5x10^4	1.5x10^4	25.82
O	Bo-eun	ME-SA/ind-2001d	1.42x10^7	1.42x10^5	1.42x10^5	18.41
O	Jeong-eup	ME-SA/Ind-2001d	3.56x10^6	3.56x10^4	3.56x10^4	19.18
O	O1manisa	ME-SA	1.12x10^6	1.12x10^4	1.12x10^4	20.2
A	Po-cheon	Asia/Sea-97	4.74x10^6	4.74x10^5	4.74x10^5	16
A	Yeon-cheon	Asia/Sea-97	1.50x10^6	1.5x10^5	1.5x10^5	16.02
A	Malaysia97	Asia/Sea-97	2.0x10^6	2.0x10^4	2.0x10^4	22.27
A	P1A-189	FMDV A/SAU/2/2015	4.74x10^5	4.74x10^4	4.74x10^4	16.38
A	Iran05	Asia/Iran-05	6.32x10^5	6.32x10^4	6.32x10^4	17.06
A	A22 Iraq	Asia/G-IV	1.12x10^6	1.12x10^5	1.12x10^4	19.6
Asia1	MOG/05	G-V	1.50x10^7	1.5x10^5	1.5x10^5	17.08
Asia1	CAM/9/80		8.43x10^6	8.43x10^4	8.43x10^4	17.56
Asia1	Shamir		1.12x10^7	1.12x10^5	1.12x10^5	20.61
SAT1	SAT1/BOT/1/68	WZ(Ⅲ)	8.43x10^6	-	8.43x10^5	15.33
SAT2	SAT2/ZIM/5/81	WZ(Ⅱ)	1.50x10^6	-	1.5x10^5	15.84
SAT3	SAT3/ZIM/4/81		7.38x10^6	-	7.38x10^4	19.05

Limit of detection (Tissue spiking)

Serotype	Strain	TCID$_{50}$/㎖	Rapid Cut-off		
			VDRG FMDV 3Difff/PAN(TCID$_{50}$/ml)		RT-PCT
			Strip 3Diff	Strip PAN	Ct value
O	O1manisa	5.01x10^7	5.01x10^4	5.01x10^4	24.94
A	A22 Iraq	3.16x10^6	3.16x10^4	3.16x10^4	24.85
Asia1	Shamir	3.2x10^5	3.2x10^4	3.2x10^4	24.76
SAT1	SAT1/BOT/1/68	2x10^6	-	2x10^5	19.33
SAT2	SAT2/ZIM/5/81	7.9x10^5	-	7.9x10^4	22.01
SAT3	SAT3/ZIM/4/81	5.01x10^6	-	5.01x10^4	24.96

Repeatability

***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 repeatability with three lots of products and found that 100% of the results were consistent.

Standard No.	#1		#2		Rate of matching
	Strip 3Diff	Strip PAN	Strip 3Diff	Strip PAN	
FMDVO-001	3+	3+	3+	3+	100%
FMDVO-002	2+	2+	2+	2+	100%
FMDVO-003	1+	1+	1+	1+	100%
FMDVA-001	3+	3+	3+	3+	100%
FMDVA-002	2+	2+	2+	2+	100%
FMDVA-003	1+	1+	1+	1+	100%
FMDVAS-001	3+	3+	3+	3+	100%
FMDVAS-002	2+	2+	2+	2+	100%
FMDVAS-003	1+	1+	1+	1+	100%
Sal-B-001	-	-	-	-	100%
Sal-B-002	-	-	-	-	100%
Sal-P-001	-	-	-	-	100%
Sal-P-002	-	-	-	-	100%
Tis-B-001	-	-	-	-	100%
Tis-B-002	-	-	-	-	100%
Tis-P-001	-	-	-	-	100%
Tis-P-002	-	-	-	-	100%

Diagnostic characteristics

Threshold determination and Diagnostic sensitivity (DSe) and specificity (DSp) 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%)

Total 98.7% sensitivity (n=1139/1154), (95% CI: 97.87% to 99.27%)

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%)

FMDV-negative tissue in Korea (RT-PCR)

Specificity in bovine: 100% (n=150/150), (95% CI: 97.57% to 100%)

Specificity in swine: 100% (n= 150/150), (95% CI: 97.57% to 100%)

Total 99.7% specificity (n= 790/792), (95% CI: 99.09% to 99.97%)

Reproducibility

Analytical reproducibility

***Conclusion*:** Using a series of products, researchers in three different laboratories tested the standard substance (O, A, Asia1 each strong, medium, weak positive sample, negative 4 samples, total 13 samples) twice a day for 5 days per Lot. The reproducibility test results were all determined to be consistent.

Three different labs tested reproducibility, and 100% of the results were consistent.

Diagnostic reproducibility

***Conclusion*:** 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.

Two different labs tested reproducibility, and 99.5% of the results were consistent.

Reference

Ku, B., Nah, J. & Ryoo, S., Sagong, M. & Kim, T. & Park, S-H. & Lee, J-W & Lee H J. & Wee, S-H. Development of rapid detection lateral flow strip kit for Foot-and-Mouth Disease virus serotypes O, A and Asia1 in clinical samples, 2017 Global FMD Research Alliance, p63, 2017

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