BIOVET
A DIVISION OF ANTECH®

Foot-and-Mouth Disease Virus Antibody Test Kit

Multi-species FMDV NSP-3B bELISA

Product code: TRM-573 (2 plates)
TRM-574 (5 plates)

- ✓ **Highly sensitive ELISA kit**
- ✓ **A shelf life of 24 months**
- ✓ **Effectively discriminates infected from vaccinated animals**
- ✓ **Efficiently detects animals infected with all FMDV serotypes**
- ✓ **Can be used in all cloven-hoofed species including cattle, goat, sheep, and pig**

INTRODUCTION

FMD is a highly contagious infection which affects cloven-hoofed domesticated and wild ruminants such as cattle, sheep, goat as well as pig. The agent responsible for this disease is the FMDV of which seven serotypes have been identified to date: O, A, C, Asia 1, SAT 1, 2, and 3. FMD is a significant constraint to international trade in live animals and animal products. The FMDV genome encodes a single polyprotein from which the different viral polypeptides are cleaved by viral proteases, including eight different non-structural proteins (NSPs). Both structural and non-structural antigens induce the production of antibodies in infected animals.

Control of the disease in FMD-free countries includes movement restrictions and a slaughter policy. However, elimination of infected and contact animals alone may not be sufficient to eradicate the virus and emergency vaccination may be considered as an additional option. Identifying animals either vaccinated or unvaccinated that have had contact with live virus, from those that have been only vaccinated against the disease is of considerable importance since both groups have neutralizing antibodies in their sera. Using traditional serological techniques, it is not possible to distinguish FMD infected animals from vaccinated animals and control authorities have limited possibilities to monitor virus presence or circulation. By contrast, blocking ELISA based on recombinant FMDV 3ABC antigen (non-structural protein) and a 3B monoclonal antibody allows differentiating infected from vaccinated animals (DIVA), depending on the purity of the vaccine.

INTENDED USE

This kit is based on an immunoenzymatic assay for the detection of antibodies against Foot-and-Mouth Disease Virus (FMDV) in bovine, ovine and porcine serum.

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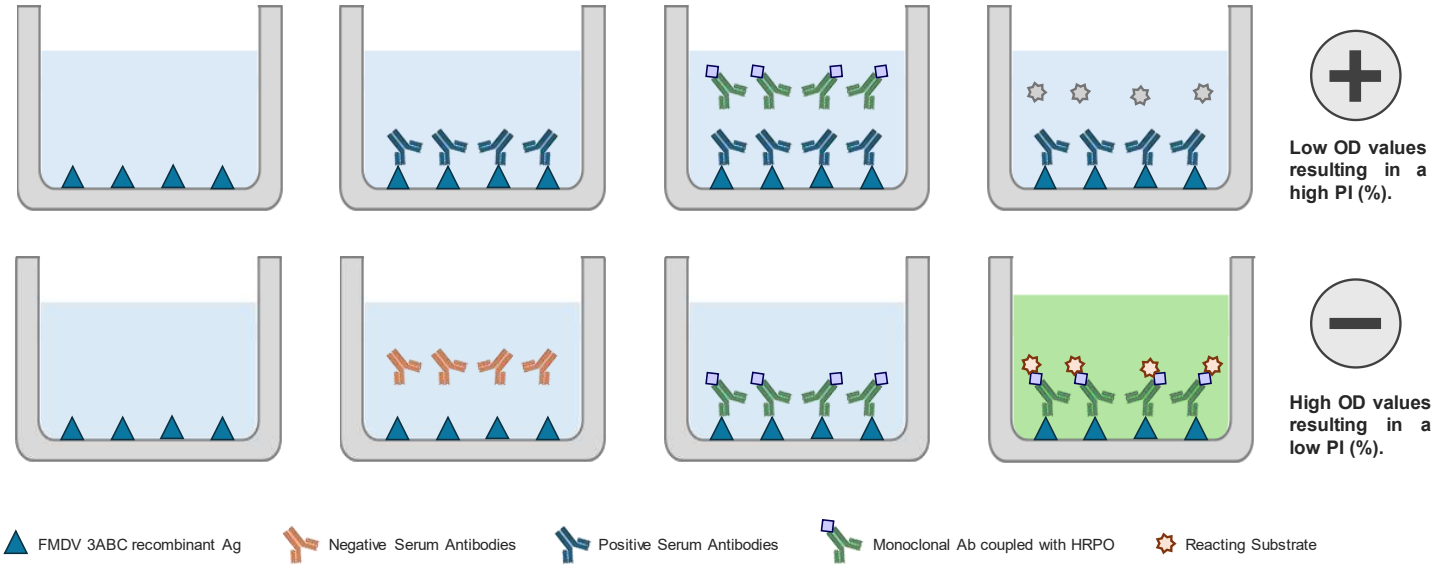
PRINCIPLE OF THE TEST

a. Assay description

1. The wells are coated with recombinant 3ABC protein of FMDV.
2. Samples are added and incubated for 1 hour at 23 ± 2°C. Each well is then washed carefully to remove unbound material.
3. An anti-FMDV 3b monoclonal antibody conjugated with HRPO is added and incubated for 30 min at 23 ± 2°C. Washing steps follow.
4. The substrate is added and incubated for 15 min at 23 ± 2°C. It will react with HRPO if present. The reaction is stopped, and OD values are measured.



b. Results interpretation

OD values are used to calculate the Percent Inhibition (PI) as follow: **PI (%) = 100 - (OD_{sample} / mean $OD_{neg.ctrl}$) * 100**

The status of a test sample is determined by the intended application. Refer to the table below.

Applications	Cattle & Sheep			Pig		
	PI cut-off	Se (%)	Sp (%)	PI cut-off	Se (%)	Sp (%)
Outbreaks of FMD (Diagnostic)	27.4	99.5	89.5	30.2	100	98.4
FMD endemic (Screening)	42.7	97.8	96.7	35.7	94.7	98.7
Absence of FMD (Monitoring)	63.5	84.5	99.4	43.8	84.2	99.3

PI: Percent Inhibition Se: Sensibility Sp: Specificity

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TECHNICAL DATA

a. Sensitivity & Specificity

Sensitivity was first assessed by observing the seroconversion of experimentally infected animals on different days post infection. Testing was carried out at the NCFAD and included:

A. serial bleeds from FMDV- infected cattle from 0 DPI to ≥ 21 DPI
B. serial bleeds from FMDV- infected sheep from 0 DPI to ≥ 14 DPI
C. serial bleeds from FMDV- infected pigs from 0 DPI to ≥ 14 DPI

A	Reference test +	-	Total
Biovet +	85	8	**93**
Biovet -	0	80	**80**
Total	**85**	**88**	**173**

B	Reference test +	-	Total
Biovet +	59	16	**75**
Biovet -	1	60	**61**
Total	**60**	**76**	**136**

C	Reference test +	-	Total
Biovet +	19	9	**28**
Biovet -	0	109	**109**
Total	**19**	**118**	**137**

Overall, the Biovet test demonstrated better detection of seroconverting samples than the reference test (PrioCHECK FMDV NS).

ROC Analysis was carried out with 1608 bovine sera (1st table) and 692 pig sera (2nd table) from North America, South America and Africa. Results are presented below:

Bovine					***Swine***				
Criterion	**Sensitivity**	***95% CI***	**Specificity**	***95% CI***	**Criterion**	**Sensitivity**	***95% CI***	**Specificity**	***95% CI***
>27.44	99.53	*98.6 - 99.9*	89.45	*87.3 - 91.3*	>30.15	100.00	*82.2 - 100.0*	98.37	*97.1 - 99.2*
>42.69	97.78	*96.3 - 98.8*	96.72	*95.4 - 97.7*	>35.69	94.74	*73.9 - 99.1*	98.66	*97.5 - 99.4*
>63.49	84.49	*81.4 - 87.2*	99.39	*98.7 - 99.8*	>43.80	84.21	*60.4 - 96.4*	99.26	*98.3 - 99.8*

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b. Limit of Detection (LOD)

Two-fold dilution series of positive sera (5 each) from bovine, ovine and porcine were tested with the Biovet kit and a Reference kit. Each dilution was tested in triplicates. The limit of detection for each kit represents the mean (15n) of the highest dilution factor from which the samples remained positive. Testing was carried out at the NCFAD.

Species	Biovet LOD	Reference LOD	Biovet / Ref.
Bovine	1 / 46.93	1 / 12.13	**3.87**
Ovine	1 / 21.87	1 / 6.80	**3.22**
Porcine	1 / 5.20	1 / 2.93	**1.77**

Based on the LOD, the Biovet kit has a better analytical sensitivity than the Reference kit for most of the sera tested.

c. Cross Reactivity

Testing was carried out at the NCFAD. The kit analytical specificity was evaluated using porcine and bovine sera positive for antibodies to:

- Seneca Valley Virus (SVA): 12 serum samples from pigs at 28 days post infection (DPI) with SVA
- Swine Vesicular Disease Virus (SVDV): 29 serum samples from pigs at 21 DPI with SVDV UKG 27/72
- Vesicular Stomatitis Virus (VSV): 14 serum samples from pigs and 4 serum samples from cattle at 28 DPI with VSV

No cross-reactivity was observed with any antiserum, giving analytical specificity of 100% for the kit.

d. DIVA Capability

Serum samples from vaccinated animals was used to evaluate the DIVA capability of the kit. Samples included:

A. Sera from 352 vaccinated cattle in South America (sera collected at 28 days or later following vaccination)
B. Sera from 16 vaccinated sheep (sera collected at ≥21 days following vaccination)
C. Sera from 51 vaccinated pigs (sera collected at ≥21 days following vaccination)

A	Reference test		
	+	-	Total
Biovet +	2	6	**8**
Biovet -	0	344	**344**
Total	**2**	**350**	**352**

B	Reference test		
	0	0	**0**
Biovet +	0	16	**16**
Biovet -	0	16	**16**
Total	**0**	**0**	**0**

C	Reference test		
	+	-	Total
Biovet +	0	0	**0**
Biovet -	0	51	**51**
Total	**0**	**51**	**51**

The Biovet test showed a great capability of differentiating infected from vaccinated individuals (DIVA capability).

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e. Stability

A panel of 12 serum samples (5 bovine, 5 porcine and 2 ovine) was tested in 3 series of the kit from serial release to 25 months at 4°C. The mean PI (%) and coefficient of variation between time point are presented bellow.

Samples	Shelf Life Final QC	13 months	19 months	25 months	*Mean*	*SD*	CV (%)
Pos. Ctrl.	96,61	96,31	96,28	96,36	*96,39*	*0,15*	**0,2**
832 - BOV	91,94	90,83	89,10	89,76	*90,41*	*1,25*	**1,4**
833 - BOV	88,00	88,14	83,65	86,35	*86,53*	*2,09*	**2,4**
835 - BOV	65,64	59,25	56,06	55,33	*59,07*	*4,7*	**8**
840 - BOV	-7,38	-10,35	-4,36	-2,31	*-6,1*	*3,52*	**N/A**
841 - BOV	-4,06	-10,49	-6,53	-9,54	*-7,65*	*2,93*	**N/A**
844 - POR	94,80	94,42	93,97	94,21	*94,35*	*0,35*	**0,4**
846 - POR	77,43	69,73	64,06	73,41	*71,15*	*5,68*	**8**
848 - POR	62,58	54,85	51,73	57,36	*56,63*	*4,59*	**8,1**
852 - POR	-0,36	-4,09	-4,04	2,86	*-1,41*	*3,34*	**N/A**
853 - POR	-8,63	-9,67	-8,04	-3,31	*-7,41*	*2,81*	**N/A**
899 - OV	85,03	82,02	82,56	83,78	*83,35*	*1,34*	**1,6**
900 - OV	12,32	9,71	8,37	10,16	*10,14*	*1,64*	**16,2**

PI (%) were highly consistent from serial release to 25 months at 4°C. Calculated CVs were below 15% for every positive sample, demonstrating the high stability of the kit. No changes in outcoming results were observed.

f. Repeatability

A panel of 8 serum samples (4 bovine, 4 porcine) was tested in triplicate within one plate, between three plates of the same serial and between three plates of three different series.

Samples	Intra-plate variability				Inter-plate variability				Inter-lot variability			
	Well 1	Well 2	Well 3	CV (%)	Plate 1	Plate 2	Plate 3	CV (%)	Serial A	Serial B	Serial C	CV (%)
Pos ctl	97,01	96,98	97,04	**0,0**	97,01	97,24	96,88	**0,2**	97,01	95,80	96,46	**0,6**
Buffer	-32,98	-33,09	-32,54	**N/A**	-32,98	-32,57	-41,83	**N/A**	-32,98	-20,80	-10,40	**N/A**
833 - BOV	88,31	89,02	88,22	**0,5**	88,31	89,38	88,76	**0,6**	88,31	86,10	86,74	**1,3**
835 - BOV	68,38	71,53	70,47	**2,3**	68,38	69,07	69,14	**0,6**	68,38	60,20	63,37	**6,4**
840 - BOV	-12,95	-1,38	-8,77	**N/A**	-12,95	1,38	-15,48	**N/A**	-12,95	-3,91	3,54	**N/A**
841 - BOV	-11,69	-7,74	-4,44	**N/A**	-11,69	-4,95	-16,18	**N/A**	-11,69	-0,72	-0,97	**N/A**
844 - POR	95,91	95,92	96,09	**0,1**	95,91	96,78	96,34	**0,5**	95,91	94,60	95,14	**0,7**
846 - POR	85,58	83,88	84,89	**1,0**	85,58	87,49	84,73	**1,6**	85,58	77,80	78,63	**5,3**
852 - POR	-1,10	11,82	9,83	**N/A**	-1,10	11,33	-5,32	**N/A**	-1,10	18,50	-3,37	**N/A**
853 - POR	-3,51	4,77	4,01	**N/A**	-3,51	3,42	-10,22	**N/A**	-3,51	-16,80	-5,26	**N/A**

Again, PI (%) were highly consistent. Calculated CVs were below 15% for every positive sample.

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CONCLUSION

The Biovet FMDV NSP-3B cELISA (multi-species) kit demonstrated excellent diagnostic performances when compared to the Reference test. The kit is highly sensitive and reliable. It can be used in all cloven-hoofed species, and it can discriminate infected animals from vaccinated ones. The kit is also extremely stable over time. It is available in 2-plates and in 5-plates format. This kit is licensed by the Canadian Centre for Veterinary Biologics for export only.

KIT COMPOSITION

Components	Quantity TRM-573	Quantity TRM-574
12 x 8 wells strips coated with recombinant FMDV 3-ABC antigen	2	5
Ready to use Positive Control	1.5 mL	3 mL
Ready to use Negative Control	1.5 mL	3 mL
Concentrated Wash Solution (10X)	2 x 100 mL	4 x 100 mL
Concentrated Conjugate (HRPO-labeled anti-3B mAb)	Variable	Variable
Ready to use Conjugate Dilution Buffer	30 mL	75 mL
Ready to use Substrate (TMB)	25 mL	60 mL
Ready to use Stop Solution	25 mL	60 mL

CFIA-licensed FEO; 710DR/F5.1/D10

For more info, contact us at: 1-888-824-6838, option 3
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