Foot and Mouth Disease Virus Antibody ELISA

AniGen FMD NSP Ab ELISA

1. Disease information

Foot-and-mouth disease (FMD) is a highly contagious viral infection primarily of cloven-hoofed domestic animals, such as cattle, pigs, sheep, goats, deer, and water buffalo. It does not affect humans. A highly contagious viral disease, it affects animals with cloven (divided) hooves. The FMD virus (FMDV) is a member of the genus *Aphthovirus* in the family Picornaviridae. There are seven serotypes of FMDV: A, O, C, Asia 1, and Southern African Territories (SAT) 1, 2 and 3. Within these serotypes, over 60 subtypes have been described, and new subtypes occasionally arise spontaneously. However, at a specific time, there are only a few subtypes causing disease throughout FMD endemic areas. The importance of subtypes is that a vaccine may have to be tailored to the subtype present in the area in which the vaccine is being used.

In many countries the disease is controlled by preventive vaccination. However, in most countries that are free of the disease, vaccination is banned. Vaccines consist of (partly) purified structural proteins of the FMD virus and therefore vaccinated animals only elicit antibodies directed against the structural proteins of the virus. However, after infection with FMDV, antibodies directed against the structural and the non-structural proteins (NSP) are produced. Therefore an ELISA detecting antibodies against NSP of FMDV detects not only infected animals but also discriminates between infected and vaccinated animals.

2. Explanation of the test

A test, which differentiates between antibodies due to vaccination from antibodies due to infection, would be of great value in the control of FMD. Several tests, which are based on NSP (specially 3 ABC protein) have been described (Berger et al., 1990, Neitzert et al., 1991, Bergmann et al., 1993, Lubroth et al., 1995). The success of the principle has also been demonstrated in pigs (Rodriguez et al. 1994). For the screening of large numbers of samples an ELISA would be highly preferable.

The AniGen FMD NSP Ab ELISA is a Competitive Enzyme Linked Immunosorbent Assay for the qualitative detection of antibody to NSP.

The AniGen FMD NSP Ab ELISA contains a microplate, which is pre-coated with recombinant 3ABC antigen on the well. For testing, ELISA plates coated with the 3ABC were incubated with an equal mixture of serum and Mab-HRP(1:100 dilution in the conjugate diluent) for 90 minutes at 37℃. During first incubation, if antibodies against 3ABC present in the test sample, the antibodies and HRP conjugated monoclonal antibodies against 3ABC competitively bind to the antigens in the well. Following this incubation, all unbound material is removed by aspiration and washing before the addition of a substrate solution. The residual enzyme activity found in the well will thus be directly inversely proportional to the anti-3ABC antibodies in serum or plasma, and evidenced by incubating the solid-phase with a substrate solution. The reaction is stopped by addition of the stop solution and colorimetric reading will be performed by using a spectrophotometer at 450nm and 620nm.

The specially selected 3ABC antigens are used as capture material in test. These enable the FMD NSP Ab ELISA to identify to FMDV outbreak antibodies in sera, with a high degree of accuracy.

3. Materials provided (480Tests/Kit)

AniGen FMD NSP Ab ELISA contains following items to perform the assay.

1) Antigen coated plate: 5 Sheets (96 wells, configured in 12 x 8 wells.)
2) Negative Control : 1 vial (1.5 ㎖) of normal bovine serum preserved in phosphate buffer with protein stabilizer. Proclin 300 (0.05%) added as preservatives.
3) Positive Control : 1 vial (1.5 ㎖) of rabbit polyclonal antibodies to FMDV preserved in phosphate buffer with protein stabilizer . Proclin 300 (0.05%) added as preservatives.
4) Washing solution (10X concentrated) : 1 bottle(250 ㎖) of PBS-Tween 20. Preservative is Proclin 300 (0.05%)
5) Enzyme Conjugate (101X concentrated) : 1 vial(1.2 ㎖) of monoclonal anti-FMDV HRP, BSA and stabilizers. Preservative is Proclin 300 (0.05%).
6) Conjugate Diluent : 1 bottle (40 ㎖) of phosphate buffered saline, BSA and stabilizers. Preservative : Proclin 300 (0.05%).
7) Substrate (Ready to use) : 1 bottle (60㎖/vial) of tetramethyl-benzidine with citrate-phosphate buffer containing H_2O_2.
8) Stopping solution : 1 bottle (80 ㎖) of 1N sulfuric acid. Ready for use.
9) 10 EA of Adhesive plate sealer.
10) Instructions for use.

4. Precautions

In order to obtain reproducible results, the following rules must be observed.

1) For in vitro diagnostic use only.
2) Do not mix reagent of different lots.
3) Use thoroughly cleaned glassware, free from contamination of metal ion or oxidating substances.
4) Use disposable gloves while handling potentially infectious material and performing the assay.
5) Substrate and stopping solution should be handled with care. Avoid contact with skin, eyes and mucous membranes. In case of accident, rinse thoroughly with running water.

5. Specimen collection and storage

1) Either fresh serum or plasma samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely. Any visible particulate matters in the sample should be removed by centrifugation at 3,000 rpm for at least 20 minutes.
2) If specimens are not immediately tested they should be refrigerated at 2~8℃. For storage periods greater than three days, freeze the specimen at -20℃ or below(serum, plasma). They should be brought to room temperature prior to use.
3) Specimens containing precipitate may yield inconsistent test results. Such specimens must be clarified prior to assaying.

6. Reagent preparation

1) Allow all reagents to come to room temperature before use.
2) Don't dilute the positive, negative and samples.
3) **Preparation of Enzyme Conjugate (101X concentrated)**: The enzyme conjugate concentrate must be diluted 1 to 100 with conjugate diluent before use.
   (Dilute 10μl stock Enzyme Conjugate in 1ml of Conjugate Diluent(1:100 dilution). Mix well.)
4) **Preparation of Washing solution (10X concentrated)**: The wash concentrated must be diluted 1 to 9 with distilled/deionized water before use. In presence of undissolved crystals, re-suspend the solution by placing the vial at 37℃ for few minutes.
   (Dilute 10ml stock Washing Solution in 90 ml of distilled/deionized water (1:9 dilution). Mix well.)

7. Procedure of the test

1) Prepare the strip wells for negative control 2 wells, positive control 2 wells and each of the samples to each well.
   Dispense 50 ㎕ of negative control into wells A1 and B1.
   Dispense 50 ㎕ of positive control into wells C1 and D1.
   Dispense 50 ㎕ of samples into the corresponding wells.
2) Dispense 50 ㎕ of diluted enzyme conjugate each wells.
3) Cover the plate(s) with an adhesive plate sealer and shake the plate(s) gently. Shaking is very important to get the reproducible results.
4) Incubate the plate(s) at 37±1℃ for 90 minutes.
5) Wash the plate(s) at 6 times with **350 ㎕** of diluted washing solution. Tap the plate(s) firmly after the last washing.
6) Dispense100 ㎕ of substrate (Ready to use) each well.
7) Incubate the wells for 15 minutes at room temperature(18~25℃).
8) Dispense 100 ㎕ of stopping solution to each well.
9) Read the absorbance of the wells with a bichromatic spectrophotometer at 450nm with reference wavelength at 620nm. Reading must be completed within 30minutes from the end of assay. Allow bubbles to dissipate before reading plate.

8. Interpretation of the test

1) Test validation

① The mean OD of the negative control must be above 0.8.

② The PI value of the positive control must be above 70.

③ If either of these values are out of range, the test result should be considered as invalid and the samples should be retested.

※If the OD_{450} of a test sample is higher than the OD_{450} max the percentage inhibition can be interpreted as 0%

If the mean OD_{450} of the negative control is below 0.800 possibly the substrate solution is too cold. In that case preheat the solution to 20~25℃ or incubate up to 30 minutes.

If the mean OD_{450} of the negative control is above 2.000 a shorter incubation period with the substrate solution is recommended.

2) PI value calculation and decision

① Calculate the mean Negative Control absorbance using the absorbance values obtained of wells A1 and B1 and then calculate the PI (Percent inhibition) value of Positive controls and each test sample using the following formula of percent inhibition

PI value = [1-(OD sample/mean OD negative)] x 100

② Based on the PI value and animal species, the samples result are interpreted as follows:

- Positive Result: PI value of sample is above 50 (i.e. ≥50.0)
- Negative Result: PI value of sample is less than 49 (i.e. <50.0)

③ For example,

- Mean OD negative control : 1.251
- OD sample : 0.868
- PI value = [1-(0.868/1.251)] x 100 = 30.6 → The sample result is interpreted as negative

9. Limitations and interferences

1) The test procedure, precautions and interpretation of results sections for this test kit must be followed closely when testing.

2) Samples

① Samples containing sodium azide may affect the test result.

② Pasteurized samples (no less than 10 hours at 60℃) may lead to diminished reactivity and therefore should not be used.

③ Heat-inactivated samples (30 minutes at 56℃) do not impair the test.

④ Anticoagulants such as heparin, EDTA, and citrate do not affect the test result.

⑤ Hemolytic samples should be centrifuged before use to avoid interference by cellular constituents.

⑥ Do not use the bacterial contaminated samples

3) Failure to add specimen in the procedure could result in a falsely negative test. Repeat testing should be considered where there is clinical suspicion of infection.

10. Storage

All reagents should be stored at 2~8℃.

11. Stability

1) Do not use after the stated expiry date.

2) Stability of once prepared reagents

Reagents	State	Storage	Stability
Working Conjugate	Once prepared	Room temp	Within 1 hour
		2 ~ 8℃	4 hours
Working Washing solution	Once prepared	Room temp.	1 weeks

12. Packaging unit

96 Tests/kit, 480 Tests/kit

13. Performance characteristics

1) Comparative detection rates in experimental infection animals

Species	Post Infection	Detection Rate (positive no./tested no.)		
		AniGen	Company A	Company B
Cattle	5 days	0/17	0/17	0/17
	7 days	41/55	44/55	39/55
	7 months	3/6	5/6	3/6
Sheep and Goat	10-12 days	6/6	6/6	6/6
	21 days	6/6	6/6	6/6
	7 months	7/10	8/10	8/10

2) Comparative detection rates in noninfected animals in contact during experimental infection

Species	Post Inoculation	Detection Rate (positive no./tested no.)		
		AniGen	Company A	Company B
Sheep and Goat	10-12 days	0/2	0/2	0/2
	21 days	2/2	2/2	2/2

3) Comparative detection rates in multiple vaccinated animals

Species	Detection Rate (positive no./tested no.)		
	AniGen	Company A	Company B
Cattle	0/115	0/27	0/115
Sheep and Goat	0/50	0/50	1/50

4) Seroprevalence rates in outbreak regions

Species	Detection Rate (positive no./tested no.)			
	AniGen	Company A	Company C	Company D
Cattle	435/563 (77.3%)	442/563 (78.5%)	253/563 (44.9%)	89/361 (24.7%)
Swine	20/214 (9.3%)	22/214 (10.3%)	14/214 (6.5%)	NT

5) Specificity in FMD free country

Species	Detection Rate (positive no./tested no.(specificity))		
	AniGen	Company A	Company D
Cattle	5/1459 (99.7%)	1/459 (99.8%)	0/78 (100%)
Swine	4/642 (99.4%)	3/342 (99.1%)	12/75 (84%)

14. Precision

Within-run and between-run precisions have been determined by the testing 10 replicates of three specimens: a negative serum, a low positive serum and a strong positive serum. The C.V (%) of negative, low positive, and strong positive values were within 10% of the time.

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