

FMDV Assay for Animal Whole Blood, Serum or Plasma Use

For Professional Use

Immunoassay for the Qualitative Detection of Antibodies to FMDV in Animal Whole Blood, Serum or Plasma

Catalog No. BSP-611
US Vet Lic No.

Intended Use

The **BioSign™ FMDV** test is an *in vitro*, qualitative, immuno-chromatographic assay for the detection of antibodies to non-structural proteins of foot-and-mouth disease virus (FMDV) in serum, plasma or whole blood.

Principle

The foot-and-mouth disease (FMD) virus, an *aphthovirus* of *Picornaviridae* family, causes a highly contagious, economically important disease in cloven-hoofed animals that affects countries with cattle-breeding. Typical cases of FMD are characterized by the formation of vesicles and epithelial erosions of snout, tongue, hard and soft palate, coronary band and feet. Serologically, the FMDV is classified into seven distinct serotypes. Routine serological diagnosis of FMD is carried out by the combined use of immunodiffusion, complement fixation, ELISA and virus neutralization assays that cannot distinguish the infected from the vaccinated animals. The ability to serologically distinguish FMD-convalescent animals from those that were vaccinated livestock was first proposed in 1966 only to realize that animals that received multiple vaccinations could be reactors.⁶ According to recent reports, assays demonstrating antibodies against nonstructural proteins have the potential to differentiate infected animals from those that have been vaccinated.^{1,2,3,4,5} These tests would be able to detect continued viral circulation and extremely useful for serological surveys with a view to eradication. Furthermore, an additional benefit of the assays using nonstructural proteins is the fact that a single test can be used to detect the exposure to the virus regardless of the serotype of virus involved. However, most of these assays are indirect ELISA format requiring and dedicated laboratory expertise, stable reagents, electricity, and multistep sample handling or preparation.

BioSign™ FMDV is a pen-side assay for FMD based on lateral flow immunoassay, which is quick and could be performed without the use of sophisticated equipment.⁷

Reagents

Materials Provided

- Each **BioSign™ FMDV** test device contains a membrane strip coated with recombinant FMDV antigens. The test kit does not contain active virus.
- Each kit contains 7 mL of Developer Solution containing 0.1% sodium azide and 10ml of Blood Dilution Solution containing 0.1% sodium azide.
- test tube for whole blood assay
- Directions for Use

Materials Required but Not Provided

- Vacutainer
- Centrifuge
- Micropipetter (0-200 µL)

Warnings and Precautions

- For *in vitro* diagnostic use only.
- Do not interchange materials from different product lots and do not use beyond the expiration date.
- All specimens should be handled as if they are capable of transmitting disease. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of specimens.
- Reagents in this kit contain sodium azide as a preservative, which may react with lead or copper in plumbing to form potentially explosive metal azides. Upon disposal, always flush with a large volume of water to prevent azide buildup in drains.
- The **BioSign™ FMDV** device should remain in its original sealed pouch until ready for use. Do not use the test if the seal is broken or the pouch is damaged.

Storage and Stability

The **BioSign™ FMDV** test kit is to be stored at 2–30 °C (36–86°F) in the sealed pouch. The storage conditions and expiration date given were established under normal laboratory conditions.

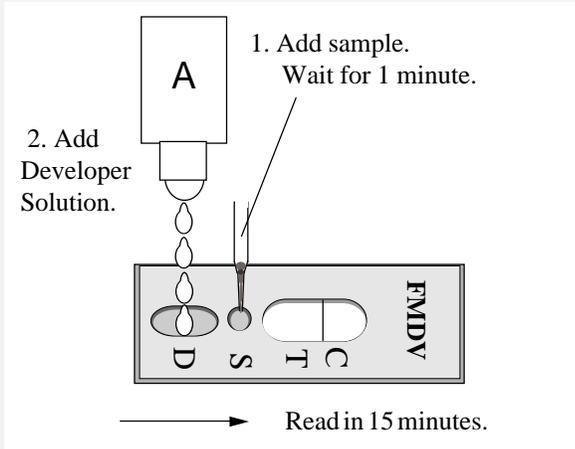
Specimen Collection and Preparation

- Collect the blood in a standard tube containing heparin or EDTA as anticoagulant. Standard clinical laboratory procedures should be used for collecting, transporting and processing specimens.
- Heat inactivation of samples may lead to hemolysis or protein denaturation, and therefore should be avoided.
- Turbid serum samples should be centrifuged for 15 minutes at approximately 1,000 relative centrifugal force.
- Specimens should be run as soon as possible. For short periods, less than 24 hours, specimens should be refrigerated at 2 to 8°C. For storage longer than 24 hours, plasma or serum should be stored at temperatures below -20°C. Do not freeze whole blood sample. If specimens are to be shipped, they should be packed in compliance with regional regulations covering the transportation of etiologic agents.
- The frozen specimens must be completely thawed, thoroughly mixed, and brought to room temperature prior to testing.

Procedure

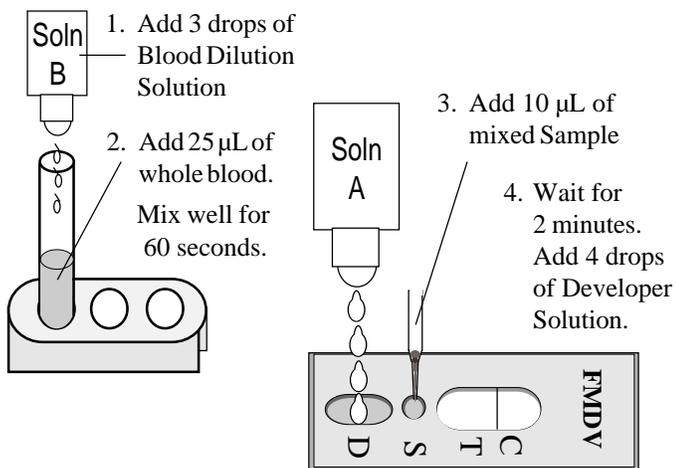
Serum or plasma assay:

1. Add 10 μL of serum or plasma sample to the Sample Well (S) and wait for 1 minute.
2. Add 4 drops (120 μL) of Developer Solution (Solution A) to the Developer Solution Well (D).
3. Read the result in 15 minutes.



Whole blood assay:

1. Dispense 3 drops of Blood Dilution Solution (**Soln B**) into test tube provided.
2. Add 25 μL of whole blood into the test tube and mix well for 60 seconds.
3. Add 10 μL of mixed sample to Sample Well (S) and allow all of sample to run up along the test strip for 2 minutes.
4. Add 4 drops of Developer Solution (**Soln A**) to Developer Solution Well (D).
5. **Add another drop of Developer Solution (Soln A) at 10 minutes** from the sample application.
6. Read the result in 10 minutes after Step 5.



5. Add another drop of Developer Solution (Soln A) at 10 minutes.
6. Read in 10 minutes after Step 5.

Procedural Notes

The instructions below must be followed to achieve optimal test performance.

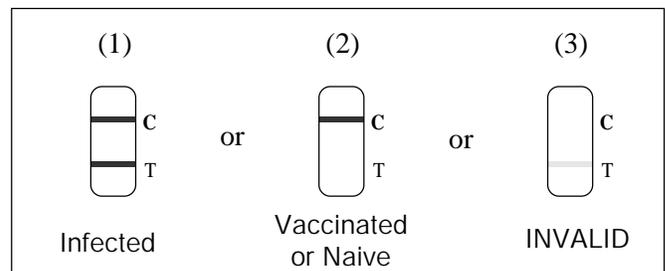
- If specimens, kit reagents or **BioSign™** devices have been stored in a refrigerator, allow them to warm to room temperature before testing.
- Do not open the foil pouch until you are ready to perform the test.
- To avoid contamination, do not touch the tip of the dropper bottle containing Developer Solution or Blood Dilution Solution with your hands or to the device.
- Label the device with the specimen or control number.
- When the specimen is dispensed using a micropipetter, allow **the tip of the micropipette to touch lightly to the pad in the Sample Well (S)** and dispense the specimen by pressing the micropipette lever.
- To add Developer solution, hold the dropper bottle in a vertical position above the Developer Solution Well and dispense 4 full drops into the well.
- After testing, dispose of the **BioSign™** device and the specimen dispenser following good laboratory practices. Consider each material that comes into contact with specimen to be potentially infectious.

Interpretation of Results

Positive: Two colored lines, one at the Test position and the other at the Control position, indicate that antibodies against NSP of FMDV have been detected. The animal was infected by FMDV.

Negative: Only one colored line at the Control position (C), with no distinct colored line at Test position, indicates that antibodies against NSP of FMDV have not been detected. The animal was uninfected by FMDV (vaccinated or naive).

Invalid: A distinctive colored line at the Control position should always appear. The test is invalid and should be repeated with a new **BioSign™ FMDV** test if no line forms at the Control position.



User Quality Control

Control standards are not supplied with this kit; however, it is recommended that controls be tested as good laboratory testing practice. Before using a new kit with specimens, positive and negative controls should be tested to confirm the test procedure, and to verify the tests produce the expected Q.C. results. Controls should also be run when there is any question concerning the validity of results obtained.

Each **BioSign™ FMDV** test device has built-in control. The Control line is an internal positive procedural control. A distinctive reddish-purple Control line should always appear if the test procedure is performed properly, an adequate sample volume is used, the sample and reagent are wicking on the membrane, and the test reagents are working.

In addition, if the test has been performed correctly and the **BioSign™** device is working properly, the background in the result window will clear and provide a distinct result. This may be considered the internal negative procedural control. If the Control line does not clear, the test is invalid and a new test should be performed. If the problem persists, contact PBM for technical assistance.

The positive and negative procedural controls contained in each **BioSign™** test device satisfy the requirements of testing a positive control and a negative control on a daily basis.

Performance Characteristics

A total of 1540 identified clinical samples were tested with **BioSign™ FMDV**. The samples comprised of negative samples prior to vaccination, vaccinated samples that were not infected, and infected samples. All tests were performed by properly trained users in random order according to the instructions given in the package inserts.

The **BioSign™ FMDV** test demonstrated a relative sensitivity of 98.6% (69/70) and relative specificity of 98.6% (1449/1470) when compared with the reference test. The overall accuracy was 98.6% (1518/1540).

Table 1

BioSign™ FMDV vs. Reference Test

		BioSign™ FMDV		
		Positive	Negative	Total
Reference Test	Infected (+)	69	1	70
	Naive (-)	11	1236	1247
	Vaccinated (-)			
	Single	4	149	153
	Multi	6	64	70
Total		90	1450	1540

References

1. Mackay, D.K.J., Forsyth M.A., Davvies, P.R., Berlinzani A., Belsham G.J., Flint M. and Ryan M.D. (1997) Differentiation infection from vaccination in foot-and-mouth disease using a panel of recombinant, non-structural proteins in ELISA. *Vaccine*, **16**, 446-459.
2. Rodriguez, A., Dopazo J., Saiz J.C. and Sobrino F. (1994) Immunogenicity of non-structural proteins of foot-and-mouth disease virus: differences between infected and vaccinated swine, *Archives of virology*, **136**, 123-131.
3. Sorensen, K.J., Madsen K.G., Madsen E.S., Salt J.S., Nqindi J.

- and Mackay D.K. (1998) Differentiation of infection from vaccination in foot-and -mouth disease by the detection of antibodies to the non-structural proteins 3D, 3AB and 3ABC in ELISA using antigens expressed in baculovirus. *Arch. Virol.*, **143**, 1461-1476
4. De Diego M., Brocchi E., Mackay D. and De Simone F. (1997) The use of the nonstructural polyprotein 3ABC of FMD virus as a diagnostic antigen in ELISA to differentiate infected from vaccinated cattle. *Arch. Virol.*, **142**, 2021-2033
5. Meyer, R.F., Babcock G.D., Newman J.F., Burrage T.G., Toohey K., Lubroth J. and Brown F. (1997) Baculovirus expressed 2C of foot-and-mouth disease virus has the potential for differentiating convalescent from vaccinated. *Journal of Virological Methods*, **65**, 33-43
6. Cowan, K.M. and Graves J.H. (1966) A third antigenic component associated with foot-and -mouth disease infection. *Virol.*, **30**, 528
7. Shin, N., Yeh M.T., Shin H., Jang K., Kang J., Sur J.H. and Lubroth J. (2002) Evaluation of a rapid serological pen-side diagnostic assay for FMD. International Pig Veterinary Society Congress 2002

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