A-5431-2

Foot and Mouth Disease Virus Antibody Test Kit, Solid Phase Immunoassay

BioSign® FMDV.Ab

Veterinary Diagnostic Use Only

Immunoassay for the Qualitative Detection of FMDV antibody in Whole Blood, Serum or Plasma of Cattle

PBM

Catalog No. BSP-611

Intended Use

The **BioSign® FMDV.Ab** test is an *in vitro,* qualitative, immunochromatographic assay for the detection of antibodies to nonstructural proteins of foot-and-mouth disease virus (FMDV) in serum, plasma or whole blood. The test kit is intended to aid in the diagnosis and early detection of current and/or past FMDV infection in the surveillance of cattle populations to identify the dormant FMDV-carrier animals, and the detection of infected animals from vaccinated animals where a vaccination program is implemented.

Principle

The foot-and-mouth disease (FMD) virus, an *aphthovirus* of *Picornaviridae* family, causes a highly contagious, economically important disease in cloven-hoofed animals. Typical cases of FMD are characterized by the formation of vesicles and epithelial erosions of nose, 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 using antibodies against nonstructural proteins have the potential to detect infected animals from the vaccinated animals.[1,2,3,4,5] These tests would be able to detect continued viral circulation and thus be 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 dedicated laboratory expertise, stable reagents, electricity, and multistep sample handling or preparation. **BioSign® FMDV.Ab** is a simple and easy to use, rapid, lateral flow, immunochromatographic assay using nonstructural proteins for early detection of antibodies directly from the blood, serum or plasma sample of cattle infected

with FMDV.[7]

Reagents

Materials Provided

- **BioSign® FMDV.Ab** test device with a membrane strip coated with recombinant FMDV antigens. The test kit does not contain active virus.
- Developer Solution (9 mL) containing 0.09 % sodium azide
- Blood Dilution Solution (7 mL) containing 0.09 % sodium azide.
- Directions for Use
- Test tube for whole blood assay (optional)

Materials Required but Not Provided

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

Warnings and Precautions

- For *in vitro* animal diagnostic use only.
- Do not interchange materials from different serials 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 azide. Upon disposal, always flush with a large volume of water to prevent azide buildup in drains.
- The **BioSign® FMDV.Ab** 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.Ab** test kit is to be stored at 2-30 °C (35-86°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. Blood may be collected in tubes without an 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 (18-30 °C) prior to testing.

Procedure

Procedural Notes:

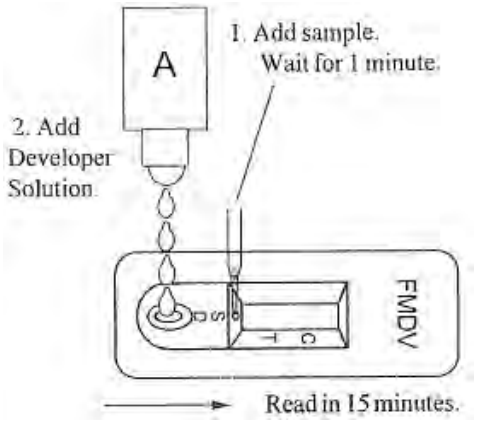
The instructions below must be followed to achieve optimal test performance.

- If specimens, kit reagents or **BioSign® FMDV.Ab** devices have been stored in a refrigerator, allow them to warm up 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 on 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 - the tip should not touch the device.
- After testing, dispose of the **BioSign® FMDV.Ab** device and the specimen dispenser pipette following good laboratory practices. Consider each material that comes into contact with specimen to be potentially infectious.

Test Procedure:

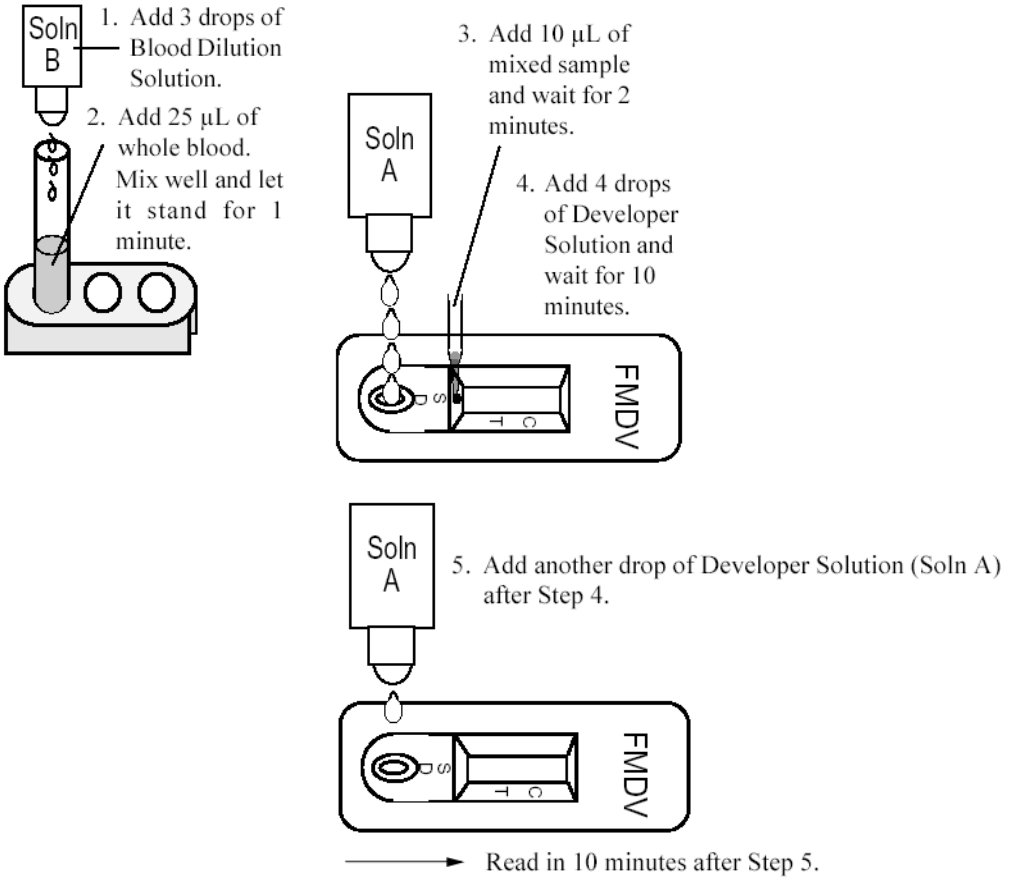
Serum or plasma assay

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 a test tube.
2. Add 25 µL of whole blood into the test tube, mix well and let it stand for 1 minute.
3. Add 10 µL of mixed sample to Sample Well (S) and wait 2 minutes for sample migration.
4. Add 4 drops of Developer Solution (**Soln A**) to Developer Solution Well (D) and wait 10 minutes.
5. ***Add another drop of Developer Solution (Soln A)*** after Step 4.
6. Read the result in 10 minutes after Step 5.



Interpretation of Results

Positive: Two colored lines, one at the Test position and the other at the Control position, indicate that antibodies against non-structural protein (NSP) of FMDV have been detected. The animal has been infected by FMDV.

Negative: Only one colored line at the Control position (C), with no distinct colored line at the Test position, indicates that antibodies against NSP of FMDV have not been detected. The animal has not been infected 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.Ab** test if no line forms at the Control position.

* Each **BioSign®FMDV.Ab** test device has a built-in control. The Control line is an internal positive procedural control. A distinct reddish-purple Control line should appear in the Control position, 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, a clear background may be considered a negative procedural control. 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.

Limitations

- The **BioSign® FMDV.Ab** test result alone should not be used for FMD diagnosis. FMDV diagnosis should be made in conjunction with clinical symptoms and/ or by viral isolation.
- A positive result suggests that antibodies to FMDV are present, which indicates active infection or exposure to the virus in the past.
- A negative result suggests that antibodies to FMDV are not present, or are present at a level below the detection limit.

Expected Values

1. The **BioSign® FMDV.Ab** test detects infection from 6 serotypes (O, A, C, Asia 1, SAT 1 and SAT 3) of FMDV. (Reactivity of SAT 2 was not confirmed due to the unavailability of sample for testing.)
2. The **BioSign® FMDV.Ab** test detects FMDV antibodies as early as 7-8 days after experimental infection in cattle. Furthermore, the test kit detects antibodies from past infection with FMDV up to 12 months after infection in cattle samples.

Performance Characteristics

A total of 899 well-characterized clinical samples were tested with **BioSign® FMDV.Ab** and commercially available ELISA. The samples were 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 results shown in Table 1 illustrate the excellent agreement between the **BioSign® FMDV.Ab** test and the reference ELISA.

Table 1. BioSign® FMDV.Ab vs. Reference Test

			BioSign® FMDV.Ab		
			Positive	Negative	Total
Reference ELISA Test	Positive	Infected (+)	69	1	70
	Negative	Naïve (-)	31	529	560
		Vaccinated (-)	17	252	269
	Total		117	782	899

Table 1 shows the result of all the tested samples combined. The **BioSign® FMDV.Ab** test demonstrated a relative sensitivity of 98.6 % (69/70) and relative specificity of 94.2% (781/829) when compared with the reference test. The overall accuracy was 94.6% (850/899).

References

1. Mackay, D.K.J., Forsyth M.A., Davies, P.R., Berlinzani A., Belsham G.J., Flint M. and Ryan M.D. (1997) Differentiating 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 animals. *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**, 528-540
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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Princeton BioMeditech Corp.

4242 US Hwy 1,

Monmouth Jct., NJ 08852, USA

Tel: (732) 274-1000

Fax: (732) 274-1010

www.pbmc.com