.

A-5921-A 1215BL

PBM

Princeton BioMeditech Corporation
P.O. Box 7139, Princeton, New Jersey 08543-7139 U.S.A.
4242 U.S. Route 1, Monmouth Junction, New Jersey 08852-1905 U.S.A.
Tel: (732) 274–1000
Fax: (732) 274–1010
Internet E-mail: support@pbmc.com
World Wide Web: http://www.pbmc.com

A-5921-A

BioSign™ FMDV Ag

New One-Step FMDV Antigens Assay

For Professional Use

Immunoassay for the Qualitative Detection of FMDV antigens in animal vesicular fluid

PBM

Intended Use

The **BioSign™ FMDV Ag** test is an *in vitro,* qualitative, one-step immunochromatographic assay for the detection of FMDV particle and 3ABC non-structural protein(NSP) in animal vesicular fluid.

Principle of Procedure

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 FMD virus 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. However, most of these assays are indirect ELISA format requiring elevated and dedicated laboratory expertise, stable reagents, electricity, and multistep sample handling or preparation.

BioSign™ FMDV Ag test is an antibody sandwich assay which detects FMD viral particle and/or 3ABC antigen in specimen from infected animals. These antigen detection tests enable detection of viral infection as early as the manifestation of signs and are extremely useful for diagnosis of FMDV infection in an outbreak situation. Because the monoclonal antibodies incorporated in the test kit to detect viral particle and 3ABC have high sensitivity to the antigens and broad specificity to the various serotypes of virus, the test kit can be used to detect the exposure to the virus regardless of the serotypes involved and all major animal species infected.

BioSign™ FMDV Ag is a pen-side assay based on lateral flow immunoassay, which is quick and easy to perform without the use of sophisticated equipment.

The **BioSign™ FMDV Ag** test uses solid-phase immunochromatographic technology for the qualitative detection of FMD viral particle and/or 3ABC antigen in vesicular fluid. The test is a two-site immunometric assay in which combinations of monoclonal antibodies are used to selectively detect FMDV antigens with a high degree of sensitivity. Each device has a Reading Window with an upper Control area and a lower Test area, and a Sample Well. In the test procedure, 15 µL of sample is added to the Sample Well and three drops of developer solution is added. The result is read within 15 minutes. If FMDV antigens are present in the specimen, it will react with the dye-conjugated antibody and bind to the immobilized antibody on the membrane to generate colored test line(s). Presence of two or more colored lines, one or two in the Test area plus a Control line, indicates a positive result, while the absence of the lines in the test window indicates a negative result.

Reagents

Materials Provided

- Each **BioSign™ FMDV Ag** test kit contains enough reagents and materials for 35 tests.
- Each **BioSign™ FMDV Ag** test device contains a membrane strip coated with anti-FMDV antibodies and a pad impregnated with antibody–dye conjugates in protein matrix containing 0.1% sodium azide; 35 test devices. The test kit does not contain active or inactive virus.
- One bottle of developer for 35 tests.
- Directions for Use.

Precautions

- For *in vitro* diagnostic use only.
- Do not use after the expiration date.

- Use a fresh transfer pipette for each specimen. Do not pipet by mouth.
- Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
- Wear disposable gloves while handling kit reagents or specimens and thoroughly wash hands afterwards.
- 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.
- The **BioSign™ FMDV Ag** 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

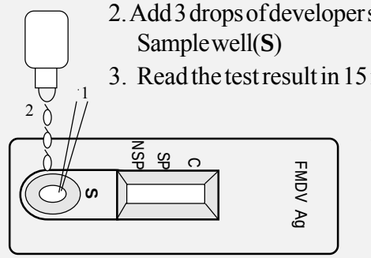
The **BioSign™ FMDV Ag** test kit should be stored at room temperature in the sealed pouch. The storage conditions and stability dating given were established under normal laboratory conditions.

Specimen Collection and Preparation

- Remove the specimen from vesicular fluid as soon as possible. Only clear specimens should be used. Specimens containing any particulate matter may give inconsistent test results. Such specimens should be clarified by centrifugation or other proper treatment before testing.
- Testing should be performed as soon as possible after sample collection. Do not leave samples at room temperature for prolonged periods.
- If specimens are to be stored, they should be refrigerated at 2–8°C or frozen. For prolonged storage, samples should be frozen and stored below –20°C. Specimens should not be repeatedly frozen and thawed.
- Bring specimens to room temperature prior to testing. The frozen specimens must be completely thawed, thoroughly mixed, and brought to room temperature prior to testing.
- If specimens are to be shipped, they should be packed in compliance with regional regulations covering the transportation of etiologic agents.

Test Procedure

1. Add 15 ul of sample in the Sample well (**S**).
2. Add 3 drops of developer solution to the Sample well(**S**)
3. Read the test result in 15 minutes.

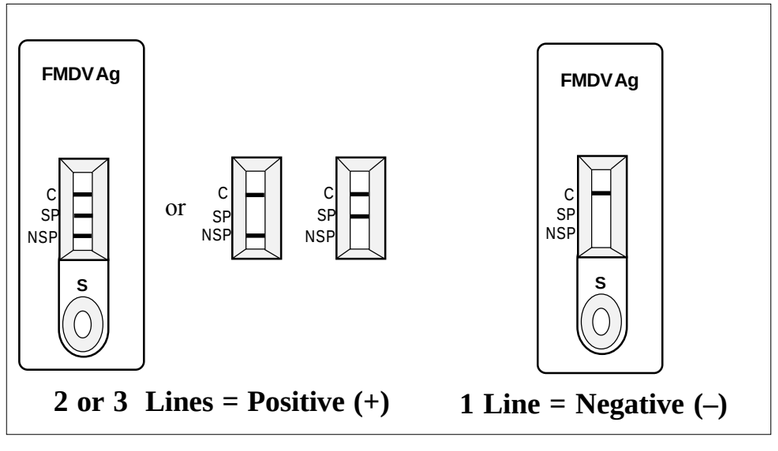


Procedure

Procedural Notes

The instructions below must be followed to achieve optimal test reactivity with the specimens. Follow the assay procedure and always perform the test under carefully standardized conditions.

- If specimens or **BioSign™** devices have been stored in the refrigerator, allow them to warm to room temperature before testing.
- Do not open the foil pouch until you are ready to perform the test.
- Several tests may be run at one time.



- To avoid cross-contamination, use a new disposable pipette for each specimen.
- Label the device with the specimen name or control number.
- 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: One Control line and one or two colored lines in the lower Test area indicate that FMD viral particle(SP line) and/or 3ABC antigen (NSP line) has been detected. The test result can be read as soon as a distinctive pink-purple line appears in the Test area. There are three possible positive cases:

a. Three colored lines, one in the SP, one in the NSP and one in the Control (C) positions. This result indicates both viral particle and 3ABC antigen are detected.

b. Two colored lines, one in the NSP and one in the Control (C) positions. This result indicates only FMDV 3ABC is detected.

c. Two colored lines, one in the SP and one in the Control (C) positions. This result indicates only FMD viral particle is detected.

Negative: Only one colored line in the Control line (C), with no distinct colored line in the Test area(SP, NSP) indicates that FMD viral particle and/or 3ABC antigen has not been detected.

***Note:** The Test line(s) will appear before the control line in most strong positive cases. TheTest line(s) may appear after the Control line in weak positive cases, and the Control line may be darker than the Test line.*

Invalid: A distinctive colored Control line should always appear. If no Control line forms after 15 minutes, the test result is invalid and new **BioSign™ FMDV Ag** test should be performed.

Limitations

- The assay must be performed in strict accordance with these instructions to obtain accurate, reproducible results.
- When performing test on a farm, a minimum of three assay kits should be tested for each sample in strict accordance with these instructions to obtain accurate, reproducible results.
- The **BioSign™ FMDV Ag** test is for *in vitro* diagnostic use only.
- This test is designed for use with vesicular fluid samples only. Use of other body fluids, including whole blood, urine, or saliva, has not been established.
- This test will indicate only the presence or absence of FMD viral antigens in the specimen, and should not be used as the only assay for the diagnosis of FMDV infection. As with all diagnostic tests, results must be considered with other clinical information available to the authorized veterinarian .
- **BioSign™ FMDV Ag** cannot detect extremely low concentrations of FMD viral particle and/or 3ABC antigen in specimens. If the test result is negative and clinical signs persist, additional follow-up testing using other clinical methods is required. A negative result at any time does not preclude the possibility of FMDV infection.

User Quality Control

A quality control (Q.C.) test using positive and negative control standards should be performed as part of good testing practice and to confirm the expected Q.C. results. The positive control will produce a moderate positive result. The negative control will yield a negative result (control line only). Upon confirmation of the expected results, the kit is ready for use with animal specimens. For information about the controls and other assistance, contact PBM's Technical Services.

A colored line in the Control window (C) can be considered an internal procedural control. If the test has been performed correctly and the device is working properly, a distinct colored line will always appear. If a test result is not clear, a new test should be performed. If the problem persists, contact PBM's Technical Services for assistance.

Performance Characteristics

BioSign™ FMDV Ag was proved to be highly sensitive and specific, when tested with *in vitro* cell culture, tongue homogenate, vesicular fluid from the infected animals at Korean National Veterinary Research and Quarantine Service(Anyang, Korea) and the Plum Island Animal Disease Center, USDA (Greenport, N.Y.).