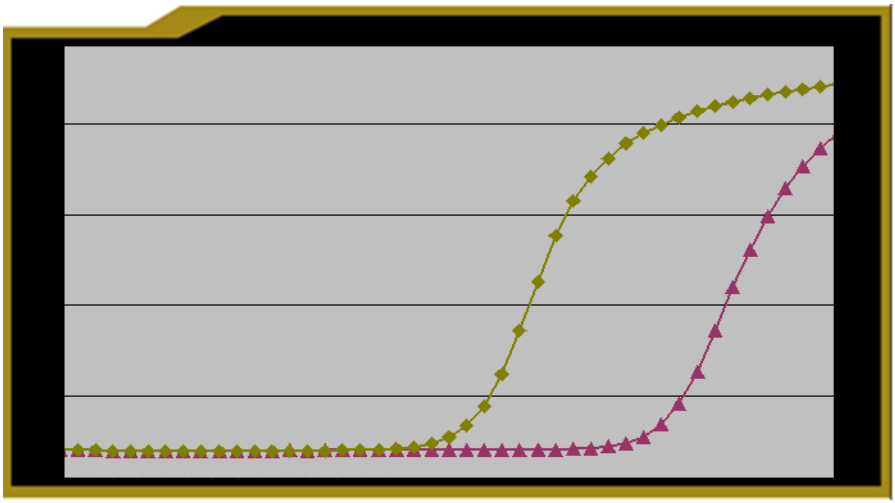
Tetracore®

VetAlert™ Foot and Mouth Disease Virus RNA Test Kit



A Diagnostic RT-PCR Test Kit for the Qualitative Detection of Foot and Mouth Disease viral RNA extracted from epithelium and serum samples from cattle, swine and sheep.

NAME AND INTENDED USE

The diagnostic reagents are intended for the rapid in vitro qualitative detection of Foot and Mouth Disease (FMD) viral RNA by real-time, reverse transcription, polymerase chain reaction (rRT-PCR). The reagents should be used to detect viral RNA extracted from epithelium and serum samples from cattle, swine and sheep. The test is intended for use by veterinary or other laboratory scientists for the presumptive identification of FMD. The test should be performed on the ABI 7500 or an equivalent real-time PCR instrument.

SUMMARY AND EXPLANATION

FMD is a highly contagious and often fatal disease of ungulate animals, affecting domestic and wild animals. FMD Virus (FMDV), the causative agent of FMD, is a member of the genus Aphthovirus belonging to the Picornaviridae family.

FMD is endemic in many countries throughout Africa, Asia and parts of South America, existing as seven distinct FMDV serotypes (A, O, C, Asia 1, Southern African Territories [SAT] 1, 2 and 3), which are distributed unevenly worldwide within seven geographically defined pools[1]. Real-time RT-PCR has been widely adopted by FMD reference laboratories as a principal tool for FMDV detection, offering high analytical sensitivity and rapid sample throughput[2,3] .

PRINCIPLES OF THE PROCEDURE

The FMD PCR test is a rRT-PCR method that utilizes a specific set of forward and reverse primers and fluorogenic probe hydrolysis chemistry for the detection of FMD viral RNA in samples. The test has a specific set of oligonucleotide primers and a FAM probe that target the 3D portion of the FMDV.

[1] Paton, D.J., K.J. Sumption, and B. Charleston, Options for control of foot-and-mouth disease: knowledge, capability and policy. Philos Trans R Soc Lond B Biol Sci, 2009. 364(1530): p. 2657-67.

[2] Reid, S.M., et al., Primary diagnosis of foot-and-mouth disease by reverse transcription polymerase chain reaction. J Virol Methods. 2000. 89(1-2): p. 167-76.

[3] Callahan, J.D., et al., Use of a portable real-time reverse transcriptase-polymerase chain reaction assay for rapid detection of foot-and-mouth disease virus. J Am Vet Med Assoc. 2002. 220(11): p. 1636-42.

PRODUCT DESCRIPTION

VetAlert™ FMD Virus RNA Test Kit

Materials Provided:

Component	Contents
Mastermix	6 Tubes of FMD Mastermix (16 reactions each)
Enzyme	1 Tube of Enzyme
Positive Control	1 Tube of FMD Positive Control
Inhibition Control	1 Tube of FMD Inhibition Control
Product Insert	1 Product Insert

Materials and Equipment Required, but Not Provided:

- RNA extraction materials
- Real-time PCR instrument: *e.g.* ABI 7500
- Micropipettes and sterile pipette tips with aerosol barriers
- 1xTE Buffer (10mM Tris-HCL, pH 8.0; 1mM EDTA) or molecular biology grade water, for use as a “no template” control (NTC)

STORAGE AND STABILITY

Store the Mastermix and Enzyme between -15°C and -25°C. Avoid repeated freeze-thaws of the reagents as this can negatively affect their performance.

Store the Inhibition Control (IC) and the Positive Control (PC) between -15°C and -25°C. To avoid repeated freeze-thaws and subsequent degradation of the IC and PC:

a. Once thawed, make small working aliquots (≥ 20 µL per tube). Use 0.5 mL sterile microcentrifuge tubes with O-rings to avoid evaporation. Store the aliquots between -15°C and -25°C. Limit each aliquot to no more than 2 freeze-thaws.

b. For short-term storage, the IC and PC can be stored between 2°C and 8°C for no more than 12 hours.

PRECAUTIONS

Real-time RT-PCR is an extremely sensitive method for amplifying and detecting small quantities of RNA. Due care should be taken to prevent any carryover contamination from previous PCR amplifications coming in contact with the reagents and positive control components in the kit.

Recommendations to prevent carryover contamination:

- Store and extract positive or suspected positive material (such as test specimens, positive extraction controls, or previously amplified material) separately from all PCR kit components, e.g., in separate rooms.
- Prepare and set-up the RT-PCR Mastermix in a BSL-2 cabinet in a DNA/RNA-free room.
- Add extracted RNA and Positive Control to reaction tubes or wells in a room separate from the Mastermix set-up.
- Use sterile pipette tips with aerosol barriers to avoid potential sample-to-sample contamination.
- Proceed with testing immediately after the addition of Enzyme to the Mastermix; do not allow the Mastermix to sit for prolonged periods before use.

LIMITATIONS

This test is used for testing RNA extracted from epithelium and serum samples from cattle, swine and sheep. No claims are made for other samples or for organisms derived from other animal species.

SPECIMEN COLLECTION AND STORAGE

Materials should be processed quickly and maintained as cold as possible during transport and laboratory accessioning to prevent loss of any FMD viral RNA that might be present. RNA must be extracted using standard methods before testing. If the samples cannot be shipped immediately, they must be stored in transport media between -60˚C and -90˚C until shipping can be arranged.

This test has been validated using the following extraction kits: Qiagen RNeasy Total RNA Isolation Kit, QIAamp RNA Blood Mini Kit and Thermo Fisher Scientific MagMAX™ Pathogen RNA/DNA Kit.

POSITIVE AND NEGATIVE CONTROLS

One PC and one “no template” control (NTC) reaction should be included with each run. The NTC is not provided, however it is recommended that a 1xTE Buffer (10mM Tris- HCL, pH 8.0; 1mM EDTA) or molecular biology grade water be used as a NTC.

Procedures

1. Preparing the Mastermix:

   a. Determine the number of reactions required.

   Note: To ensure that there is enough Mastermix prepared, an additional reaction should be added to the number of reactions required.

   b. Option A: IC is not desired, or is used as an extraction control

      i. If IC is not desired, do not add IC.

      ii. If IC is desired to serve as an extraction control, incorporate the IC into the RNA extraction process by adding 6.0 µL per sample of the IC to the extraction kit's lysis buffer (extraction kit is not provided). The product at the end of the extraction process will contain the extracted IC.

      iii. If less than 16 reactions are required, use the following table to calculate the volume of Mastermix and Enzyme needed.

1 reaction	
FMD Mastermix	19.25 µL
Enzyme	0.75 µL
Total Mastermix volume	20.00 µL

      iv. If 16 reactions are required, one Mastermix vial (16 reactions per vial) can be prepared by adding 13.5 µL of Enzyme directly to the Mastermix vial.

      v. Remove Mastermix vial(s) from the freezer. Thaw at room temperature. Once thawed, keep the Mastermix cold at all times. Gently vortex and briefly centrifuge the vials before use.

      vi. Remove Enzyme vial from the freezer and briefly centrifuge (do not vortex). Return Enzyme vial to the freezer immediately after use.

      Important: Once the Enzyme is added to the Mastermix, the prepared Mastermix must be kept on ice and used within 2 hours.

      vii. Continue to Step 2.

c. Option B: IC is used as an inhibition control for the PCR reaction

i. If less than 16 reactions are required, use the following table to calculate the volume of Mastermix, IC, and Enzyme needed.

1 reaction

FMD Mastermix	19.00 µL
IC	0.25 µL
Enzyme	0.75 µL
Total Mastermix Volume	20.00 µL

ii. If 16 reactions are required, one Mastermix vial (16 reactions per vial) can be prepared by adding 4.5 µL of IC and 13.5 µL of Enzyme directly to the Mastermix vial.

iii. Remove Mastermix vial(s) and the IC vial(s) from the freezer. Thaw at room temperature. Once thawed, keep the Mastermix and IC cold at all times. Gently vortex and briefly centrifuge the vials before use.

iv. Remove Enzyme vial from the freezer and briefly centrifuge (do not vortex). Return Enzyme vial to the freezer immediately after use.

Important: Once the Enzyme is added to the Mastermix, the prepared Mastermix must be kept on ice and used within 2 hours.

v. Continue to Step 2.

2. Aliquot 20.0 µL of the prepared Mastermix into each PCR reaction tube or well of a 96-well plate.

Note: Keep the Mastermix cold at all times to ensure optimal performance of the reagents. Use cold blocks for PCR tubes or for 96-well plates.

3. Prepare the negative control, extracted sample, and positive control reactions.

Note: Always prepare and cap the negative control first before handling either an extracted sample or the positive control.

a. Negative Control:

   Add 5.0 µL of the negative control (not provided) to a PCR reaction tube or well that contains 20.0 µL of the prepared Mastermix.

   Note: Molecular biology grade water or 1X Tris-EDTA (pH 8.0) can be used as a negative control.

b. Extracted Sample(s):

   Add 5.0 µL of the extracted sample to a PCR reaction tube or well that contains 20.0 µL of the prepared Mastermix.

c. Positive Control:

   Add 5.0 µL of the FMD Positive Control to a PCR reaction tube or well that contains 20.0 µL of the prepared Mastermix.

4. Cap and briefly centrifuge the PCR reaction tube(s) or 96-well plate.

   Note: After centrifuging, ensure that all of the mix is at the bottom of each tube or well. Centrifuge again if needed.

5. Load the PCR reaction tube(s) or 96-well plate into the appropriate real-time PCR instrument and follow the thermal cycling protocol below.

Real-Time PCR Set Up:

The assay utilizes different reporter dyes to distinguish between targets (see table below). Select the appropriate channel on the desired real-time PCR instrument to view the amplification signal for each target.

Target	Reporter Dye
FMDV	FAM
IC	Cy5 equivalent

Please call for technical assistance for questions about instrument compatibility and analysis settings.

THERMAL CYCLING PROTOCOL:
Stage 1: 48ºC for 15 minutes
Stage 2: 95ºC for 2 minutes
Stage 3: 45 cycles (2-step PCR):
Step 1: 95ºC for 10 seconds
Step 2: 60ºC for 40 seconds (collect data step)

REACTION VOLUME: 25 µL

ESTIMATED RUN TIME: 1 hour and 26 minutes (on the ABI 7500 instruments. Time may vary depending on instrument used).

ABI 7500 Settings:
Use ABI 7500 software version 1.4 or 1.5.

- If using the ABI 7500 Fast System, select Run Mode: Standard 7500.
- Do not select ROX as Passive Reference Dye. The Mastermix does not contain ROX.
- Assay: Standard Curve (Absolute Quantification)
- Target: Select detector for FAM as reporter dye and None as quencher.
- Inhibition Control: Select detector for Cy5 as reporter dye and None as quencher.
- Sample Volume: 25 µL
- Data Collection: 60°C for 40 seconds step.

Analysis Settings for the ABI 7500®

- When the run is complete, view each well individually to verify true amplification.
- Select the Amplification Plot tab to view the amplification curves. From the Tools menu, click on Graph Settings. Change Post Run Settings to Linear Y-axis and select Auto Scale.
- Adjust baseline manually as necessary for each channel.
- For each channel, set Threshold at 3% of the final normalized fluorescence of the Tetracore Positive Control amplification curve.

  Example: In the FAM channel, the final PC fluorescence at cycle 45 is approximately 1.8 and 3% of 1.8 is 0.054. Therefore, set the threshold for the FAM channel at 0.054. Round to the nearest hundredth or thousandth as appropriate.

Verify the following prior to interpretation of the results:

1. The amplification curve of the Positive Control provided has a FAM cycle threshold (Ct) value ≤ 31.
2. The NTC does not cross the threshold and remains negative until the assay endpoint is reached (45 cycles).
3. If the IC was used, the Negative and Positive Control reactions have a Cy5 Ct value ≤ 34.

Interpretation of the results

The test is a fluorogenic probe hydrolysis assay that produces a characteristic fluorescent signal with a positive test. The ideal shape of a positive amplification curve is visualized on a linear scale as a sigmoidal curve on a two-dimensional linear grid where the x-axis represents the PCR cycle number and the y-axis represents the relative fluorescence of the signal.

1. A FAM Ct value is present:

   - The result is positive for FMD if it has a Ct ≤ 40.

   - Here, a Cy5 Ct value may be dispensable, because very high concentrations of FMDV RNA may compete with the IC, producing a late Cy5 Ct value or no Cy5 Ct value at all.

2. A positive Cy5 (IC) Ct value and no FAM Ct value is measured:

    - This is a Negative Result.

    - PCR inhibition may be present if the IC Ct value is >34. If PCR inhibition is present, it is recommended that RNA extraction be repeated, or repeat the test with a diluted sample (e.g. 1:5 dilution with molecular biology grade water)

3. Neither FAM nor Cy5 Ct values are detected:

    - The test is not valid and no diagnosis is possible.

    - The PCR was inhibited and it is recommended that RNA extraction be repeated, or repeat the test with a diluted sample (e.g. 1:5 dilution with molecular biology grade water).

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