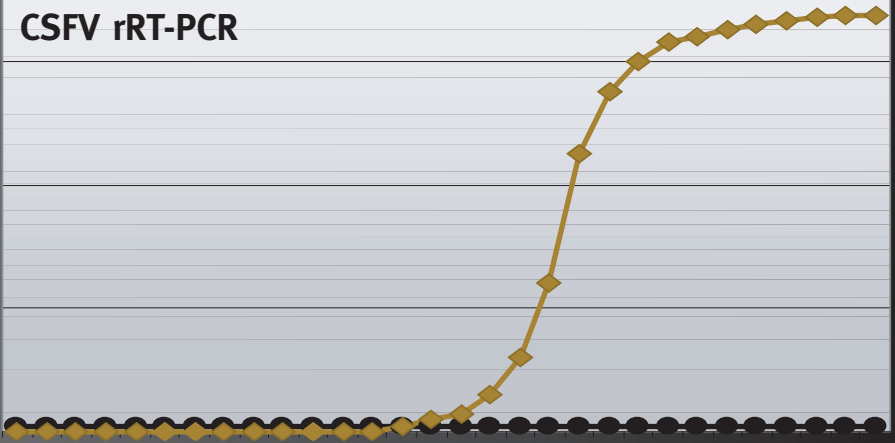


# Classical Swine Fever Virus RNA Test Kit Polymerase Chain Reaction

**VetAlert™**  
**CSFV rRT-PCR**



**A Diagnostic Test Kit for the qualitative detection of  
Classical Swine Fever Virus RNA extracted from  
porcine nasal swabs.**

## NAME AND INTENDED USE

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The diagnostic reagents are intended for the rapid in vitro qualitative detection of Classical Swine Fever (CSF) viral RNA by real-time, reverse transcriptase, polymerase chain reaction (rRT-PCR). The reagents should be used to detect viral RNA extracted from nasal swabs. The test is intended for use by veterinary or other laboratory scientists for the presumptive identification of CSF. The test should be performed on the Cepheid, SmartCycler® (versions I & II) instrument.

## SUMMARY AND EXPLANATION

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Classical Swine Fever (CSF) is a highly contagious and often fatal disease of swine, affecting domestic and wild pig populations. CSF Virus (CSFV), the causative agent of CSF, is a member of the genus Pestivirus belonging to the Flaviviridae family. Other important animal pathogens within the genus Pestivirus are Bovine Viral Diarrhea Virus (BVDV) of cattle and Border Disease Virus (BDV) of sheep, both of which can naturally infect pigs. Antibodies generated during these infections cross-react with CSFV in serologic assays making CSF diagnosis problematic (Risatti et al, 2003).

Clinical signs of CSF can remain undetected particularly during infections with CSFV strains of low virulence. Moreover, gross lesions observed at necropsy are diverse and often not pathognomonic. Rapid and precise detection of CSFV is critical for disease containment. Current diagnostic methods including detection of viral antigen in tonsils using fluorescent antibodies (FAT), or antigen capture enzyme linked immunosorbant assay (ELISA), and detection of genomic RNA by RT-PCR are relatively rapid diagnostic tests. However, these techniques require centralized laboratory facilities, and clinical specimen submissions that might delay disease diagnosis, thus affecting the efficiency of emergency disease management measures (Risatti et al, 2003). The Tetracore rRT-PCR test for detection of CSFV RNA was designed in a real-time format to overcome the limitations of classical identification methods including virus isolation and gel-based RT-PCR.

## PRINCIPLES OF THE PROCEDURE

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The assay to detect CSF viral is a real-time RT-PCR method that utilizes a specific set of forward and reverse primers and fluorogenic probe hydrolysis chemistry for the detection of CSF viral RNA in samples. The assay has a specific set of oligonucleotide primers and a FAM/TAMRA probe that target the 5'-UTR of CSFV.

## PRODUCT DESCRIPTION

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### VetAlert™ CSFV rRT-PCR

#### Materials Provided:

Component	Contents
Cepheid, SmartCycler® tube with Dried (CSF) RT-PCR Assay	rRT-PCR tests
rRT-PCR Rehydration Buffer	Buffer and dH <sub>2</sub> O
Positive Control	Plasmid, mid level positive control (approximately 2.2e+05 copies per 2.5µl)
Product Insert	1

#### Materials and Equipment Required, but Not Provided:

- RNA extraction materials
- Cepheid, SmartCycler®, version I or II
- Micropipettes and sterile pipet tips with aerosol barriers
- Microfuge with adaptor for Cepheid, SmartCycler® tubes
- 1xTE (10mM Tris-HCL, pH 8.0; 1mM EDTA), for use as a “no template control (NTC).”

## STORAGE AND STABILITY

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The dried reagents should be stored between (15°C and 30°C). The rehydration buffer should be stored between (2°C and 8°C), and the positive control should be stored at (-15° to -25°C).

## PRECAUTIONS

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Real-time PCR is an extremely sensitive means for amplifying and detecting small quantities of RNA. Due care should be utilized to prevent any carryover contamination from previous PCR amplifications coming in contact with mastermix and polymerase in this 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.
- Rehydrate the dried rRT-PCR mastermix in a BSL-2 cabinet in a DNA-free room.
- Add extracted DNA and positive control DNA to reaction tubes or wells in a room separate from the DNA-free room used for rehydration.
- Use sterile pipette tips with aerosol barriers to avoid potential sample-to-sample contamination
- Proceed with testing immediately after rehydration of mastermix; do not allow the rehydrated mastermix to sit for prolonged periods before use.
- The positive control should be stored between -15°C and -25°C for long term storage and can be stored up to two weeks at 2°C and 8°C. Avoid repeated freeze-thaw cycles of the positive control (>2x) as this may lead to degradation of the oligonucleotide control.
- The threshold setting on the Cepheid setting must be set manually, see the section entitled: Cepheid, SmartCycler® Settings
- Color indicating Desiccants Packs are placed in the pouch containing dried tests to protect the tests from exposure to humidity. Under normal conditions, the desiccant pack should be blue in color. Should the color be pink, this indicates that the pouch has been damaged and the tests should not be used. Please contact Tetracore, Inc., should such a condition be noticed.

## LIMITATIONS

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This test is for used for testing RNA extracted from swine nasal swabs samples. No claims are made for other samples or for organisms derived from other animal species.

This formulation is specially prepared for use on the Cepheid, SmartCycler®, versions I & II.

## SPECIMEN COLLECTION AND STORAGE

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Materials should be processed quickly and maintained as cold as possible during transport and laboratory accessioning to prevent loss of any CSFV RNA that might be present. RNA must be extracted using standard methods (Risatti, et al, 2003) before testing. Briefly, Dacron swabs used for collection of swine nasal swab samples must be placed in a transport tube containing 1.5 ml of transport media (DMEM with antibiotics and antifungals) and sent by overnight delivery in a cold shipping container with frozen ice packs. If the nasal swabs cannot be shipped immediately, they must be stored in transport media at -70°C, range (-60° to -90°C) until shipping can be arranged.

This test has been validated using the Qiagen RNeasy Total RNA Isolation Kit, Catalogs #74104 and #74106 (Qiagen, Valencia, CA).

## POSITIVE AND NEGATIVE CONTROLS

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

## PROCEDURE

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1. Remove desired number of tubes from pouch and place into racks.
2. Add 22.5µl of rehydration buffer to each tube.
3. Add 2.5µl of extracted material or control to each tube.
4. Cap each tube until the snap is heard.
5. Briefly centrifuge each tube in a microfuge that accommodates the Cepheid, SmartCycler® tubes.
6. Pinch each tube 8-10 times to assure that the material is well mixed, as you pinch the tube you will note the rise and fall of the meniscus (liquid) within the tube.
7. Place into the Cepheid, SmartCycler®.

## CYCLING CONDITIONS

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1. RT step: 600 seconds @ 60°C
2. 2-step PCR: (95°C x 2 seconds, 60°C x 30 seconds) for 45 cycles

### Cepheid, SmartCycler® Settings

The threshold setting must be set manually by first selecting Analysis Setting on the Cepheid, SmartCycler®. The manual threshold fluorescent units can then be changed from the default setting of 30 units to 50 units for the FAM channel.

Version I - FTTR25-Dye Set, 25µL reaction with channel 1 as FAM dye, channel 2 as Tet dye, channel 3 as Tamra dye, channel 4 as Rox dye.

Version II - FTTC25-Dye Set, 25µL reaction with channel 1 as FAM dye, channel 2 as Tet dye, channel 3 as Texas Red (TxR) dye, channel 4 as Cy5 dye.

## INTERPRETATION OF TEST RESULTS

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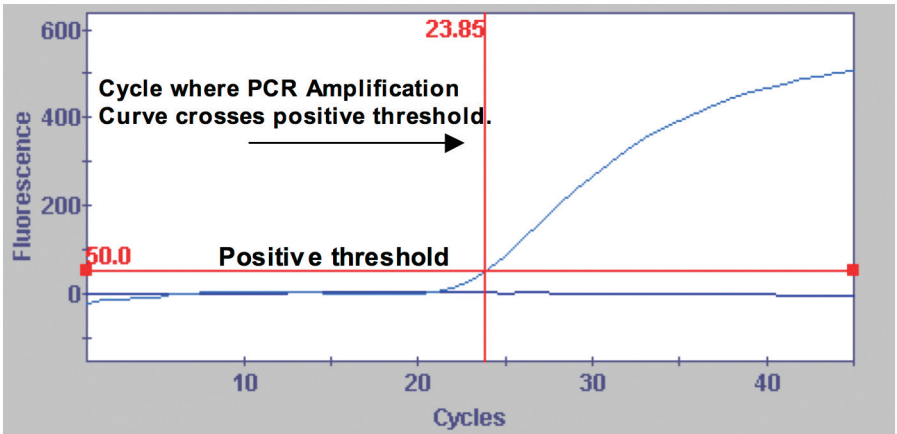
The test is a fluorogenic probe hydrolysis assay that produces a characteristic fluorescent signal with a positive test. The ideal shape of a positive amplification curve is visualized on a linear scale as a sigmoidal curve on a two-dimensional linear grid where the X-axis represents the PCR cycle number and the Y-axis represents the relative fluorescence of the signal (Figure 1).

A run is considered valid if:

- a. The amplification curve of the positive control provided must have a cycle threshold (Ct) value that crosses the threshold less than or equal to cycle 29.5.
- b. The NTC must not cross the threshold and remain negative until the assay endpoint is reached (45 cycles).

A sample is considered initially positive if the amplification curve crosses the threshold at cycle 45 or earlier and should be retested in duplicate. After retesting, the sample is considered positive if at least one of the duplicate tests is positive. Positive samples must be confirmed by virus isolation and serological tests at the USDA-APHIS-FADDL.

**Figure 1.** Seen below are typical amplification curves generated by the test on the Cepheid, SmartCycler® instrument. The ideal amplification curve is sigmoidal in shape on a linear graph. Shown are typical amplification curves from the positive control (provided) and the no-template control (NTC, not provided). The red vertical lines indicate the cycle number at which the amplification curve crosses the positive threshold, which is manually set to 50.





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