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**Protocol of fluorogenic RT-PCR for swine vesicular disease
virus**

猪水泡病病毒荧光RT-PCR检测方法
(English Translation)

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Table of Contents

Foreword
1 Scope
2 Acronyms
2.1 Fluorescent RT-PCR
2.3 RNA
2.4 DEPC
2.5 PBS
2.6 T Rao enzyme q
2.7 S D Vp Vp
4 Materials and Reagents
4.1 Instruments and Equipment

Foreword

The standard reference to the OIE (OIE) "Terrestrial Animals Manual of diagnostic tests and vaccines (mammals, birds and bees)" (5th edition).

Appendix A of this standard is a normative appendix.

The standard proposed by the People's Republic of China Ministry of Agriculture.

This standard by the National Standardization Technical Committee on Animal Epidemic Prevention.

This standard was drafted. People's Republic of China SZCIQ, People's Republic of China Yunnan Entry-Exit Inspection and Quarantine Bureau.

1 Scope

This standard specifies the method of operation swine vesicular disease virus fluorescent RT-PCR detection.

This standard applies to animals and their products in the detection of swine vesicular disease virus.

2 Acronyms

The following abbreviations apply to this standard.

2.1 Fluorescent RT-PCR

Fluorescence reverse transcription - polymerase chain reaction.

The number of cycles when fluorescence signal of each reaction tube reaches a set threshold experienced.

2.3 RNA

RNA.

2.4 DEPC

Dicarbonate phospholipids.

2.5 PBS

Phosphate buffered saline, recipe see Appendix A.

2.6 T Rao enzyme q

T Rao q DNA polymerase.

2.7 S D Vp Vp

Swine vesicular disease virus.

Principle 3

According to a specific gene sequence of swine vesicular disease virus synthesized a pair of specific primers and a dual-labeled probes specific fluorescence. Primers and probe design and through rigorous screening to cover all reported strains of swine vesicular disease virus. 5' end fluorescent probes labeled FAM Fluorescein, 3' TAMRA labeled fluorescein, which can absorb at close range within the 5' fluorophore reporter fluorescent signal emitted. Amplification, Since T Rao q enzyme 5' → 3' exonuclease activity, when extended to the fluorescent probe, which was cut to two groups separated, quenching effect disappears, fluorescent letter Number generation. Thus, by detecting the fluorescence signal is detected on a nucleic acid template.

4.1 Instruments and Equipment

4.1.1

RT-PCR fluorescence detector.

4.1.2

high-speed desktop refrigerated centrifuge (centrifugal speed 12000r/min or more).

Palm Desktop 4.1.3 or centrifuge (centrifugal speed 3000r/min).

4.1.4

Mixer.

4.1.5

refrigerator (2 °C ~ 8 °C and -20 °C two kinds).

4.1.6

Micro adjustable pipette (5μL, 10μL, 100μL, 1000μL) and supporting filter tips.

4.1.7 1.5mL, 0.5mL siliconized Eppendorf tube. the Eppendorf tubes and emitters containing 0.1% DEPC soaked in distilled water three Overnight, 121 °C ± 2 °C autoclave 15min, 40 °C drying spare. Commercially available Eppendorf tubes and emitters have been suicide, can be directly