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**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB/T 22916-2008

Protocol of fluorogenic RT-PCR for vesicular stomatitis virus

水泡性口炎病毒荧光RT-PCR检测方法

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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, Appendix B is an informative annex. 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. The main drafters of this standard. flower Qun Yi, Zhou Xiaoli, Zeng Ling, Caochen Fu, Zhanai Jun, Zhang Caihong, Lin Qingyan, Chen Bing, Yang Yunqing, Sun Jie. Vesicular stomatitis virus fluorescent RT-PCR detection method

1 Scope

GB/T 22916-2008 is the Chinese national standard on protocol of fluorogenic rt-pcr for vesicular stomatitis virus, in the field of health care technology. The /T suffix marks it as a recommended standard: it is not compulsory by itself, but it becomes binding as soon as a contract, a tender or a customer specification calls it up - which in practice is how most foreign buyers meet it. It was issued on 31 December 2008 by the General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China; Standardization Administration of China, and has been in force since 1 May 2009. Classification: ICS 11.220, CCS B41. This page is published from the official record of the standard held by the Chinese standards administration: the identification, the dates, the classification and the issuing body are taken from there. The clause text, the tables and the numeric limits are in the document itself, which is delivered complete in English translation.

This standard specifies the method of operation of vesicular stomatitis virus fluorescent RT-PCR detection. This standard applies to animals and their products in detecting vesicular stomatitis 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.

2.4 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 Vp Vp S Vesicular stomatitis virus. Principle

3 Vesicular stomatitis virus is a RNA virus, according to a specific gene sequence of a total of two types of vesicular stomatitis virus, A pair of specific primers And a fluorescent substance-specific dual-labeled probes. Through rigorous design and selection of primers and probes, covering two vesicular stomatitis virus Type and mutant strains of vesicular stomatitis virus-specific universal primers and probes. Probe 5 'end FAM labeled fluorescein fluorescence emitted by it Light can be received detection equipment, the report called fluorophores; 3 'TAMRA labeled fluorescein, which can absorb at close range within the 5' end report Fluorophore fluorescence signal emitted, called quenching fluorophore. When amplification, T Rao q enzyme plays its 5 '-> 3' exonuclease end functions Can the probe into a single nucleotide hydrolysis, eliminate obstacles, the report marks both ends of the fluorophore and quencher probe fluorophores are free in solution , The instrument detects the fluorescent signal emitted.

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).

4.1.3 tabletop centrifuge (centrifugal speed 3000r/min).

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.