

ICS 65.020.01
CCS B 16

SN

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

SN/T 5197-2019

**Operation protocol of real-time quantitative PCR for bovine
sarcoïdosis virus
(English Translation)**

Issued on: January 1, 1970

Implemented on: July 1, 2020

Issued by: General Administration of Customs

Table of Contents

Foreword	
1 Scope	
2 Normative references	
3 Abbreviations	
4 Principle	
5 Equipment and reagents	
5.1 Main instruments and equipment	
5.2 Reagents and consumables	
5.3 Standard strains and cells	
6 Preparation of control samples	
6.1 Positive control sample	
6.2 Negative control sample	
7 Operation method	
7.1 Handling of samples	
7.2 Extraction and purification of viral DNA	
7.3 Fluorescence quantitative PCR detection	
8 Result judgment	
8.1 Result analysis	
8.2 Quality control standards	
8.3 Judgment of results	
35.0 Appendix A	

Foreword

This standard was drafted in accordance with the rules given in GB/T 1.1-2009.

This standard was proposed and managed by the General Administration of Customs of the People's Republic of China.

Drafting organization of this standard. Chongqing Customs of the People's Republic of China.

1 Scope

This standard specifies the fluorescent quantitative PCR detection method for bovine nodular herpes virus.

This standard is applicable to the detection of bovine nodular herpes virus nucleic acid.

2 Normative references

The following documents are indispensable for the application of this document. For dated reference documents, only the dated version applies to this document. For undated reference documents, the latest version (including all amendments) is applicable to this document.

GB/T 6682 Analytical laboratory water specifications and test methods GB 19489 Laboratory Biosafety General Requirements GB/T 27401 Laboratory Quality Control Specification Animal Quarantine GB/T 27403 Laboratory Quality Control Specification Food Molecular Biology Testing

3 Abbreviations

The following abbreviations apply to this document.

Bp. base pair

CPE. cytopathic effect Ct. The number of cycles experienced when the fluorescence signal in each reaction tube reaches the set threshold (cycle threshold) DNA. deoxyribonucleic acid LSDV. lumpy skin disease virus PBS. phosphate buffered saline PCR. polymerase chain reaction (polymerase chain reaction) Taq enzyme. DNA polymerase (TaqDNA polymerase)

4 Principle

The Taq Man fluorescent quantitative PCR method was used to compare the conservation of bovine nodular herpes virus, goat pox virus and ovine pox virus Gene, design a pair of specific primers that are only conserved in the bovine nodular herpes virus gene and a specific fluorescent double label for the ITR region Remember the MGB probe. The binding site of the probe is located inside the target amplified fragment. The 5'end labeled FAM fluorescein is the reporter fluorescence the group (R), 3'-labeled MGB fluorescein (Q) can absorb the fluorescent signal emitted by the 5'-end fluorophore in a short distance, which is called to quench the fluorescent group. When the reaction is annealed, the primers and probes bind to the target gene fragment at the same time, and the fluorescence emitted by the R group on the probe the light signal is absorbed by the Q group, and the instrument cannot detect the fluorescent signal; and when the reaction proceeds to the extension

stage, the Taq enzyme exerts 5'→3' the function of exonuclease, which degrades the probe. In this way, the R group on the probe is freed, and the emitted fluorescence is no longer absorbed by Q it is received by the detector. As the PCR reaction progresses, the PCR product and the increase of the fluorescence signal show a corresponding relationship.

5.1 Main instruments and equipment

5.1.1

Fluorescence quantitative PCR instrument.

5.1.2

Low temperature high speed centrifuge, micro high speed centrifuge.

5.1.3

Low-temperature refrigerator.

5.1.4

Micro oscillator.

5.1.5

Biological safety cabinet.

5.1.6

Constant temperature water bath.

5.1.7

Autoclave.

5.1.8

Micro adjustable pipette.

5.2 Reagents and consumables

5.2.1

Nutrient solution. see A.1 for the preparation method.

5.2.2

Maintenance solution. see A.2. for the preparation method.

5.2.3

Cell dispersion. see A.3 for the preparation method.

5.2.4

Commercialized trace virus nucleic acid extraction kit or other commercialized kits.

5.2.5

Premix Ex Taq (Probe qPCR) fluorescent PCR buffer.

5.2.6

Primer. According to the gene sequence of the international standard strain of bovine nodular herpes virus (LSDV), design and synthesize in its most conservative ITR region sequence A pair of specific primers, F. 5'-TTGTCAGAAACGAGG-3', R.

5'-ATGCCTCACTTGTATTTGG-3' and a MGB the probe

[(FAM)5'-TCTTGCTAAAATACCA-3'(MGB)] is prepared at 10 µmol/L and stored at -20°C.

Note: The reagents and water used above are all analytical reagents unless otherwise specified, and the water should meet the specifications of Grade 3 water specified in GB/T 6682.

5.3 Standard strains and cells

5.3.1

Standard strain. The Neethling strain, an international standard strain of LSDV, is used as the reference strain for the test.

5.3.2

Cells. Take healthy lamb testes according to conventional methods to prepare primary testicular cells; or use MDBK cells or Vero cells.

6.1 Positive control sample

Provided by the bovine nodular skin disease reference laboratory or prepared according to the following method. the bovine nodular eruption international standard strain (5.3.1)

Inoculate primary testicular cells (5.3.2) at 10%, absorb at 37°C for 1 h, add maintenance solution (5.2.2), and incubate at 37°C with 5% CO₂.

Harvest the virus suspension when the CPE reaches 70%. freeze and thaw 3 to 4 times, centrifuge at 5,000 r/min for 10 min, and take the supernatant for use. also may A plasmid containing the DNA sequence of the amplified region of LSDV ITR was used as a positive control.

6.2 Negative control sample

Provided by the bovine nodular skin disease reference laboratory or prepared according to the following method. freeze-thaw the well-growing primary testicular cells Centrifuge at 5,000 r/min for 3 to 4 times for 10 min, and take the supernatant for use.