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Quarantine protocol for real-time PCR of Lawsona intracellularis

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(English Translation)

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Foreword

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

Please note that some of the content of this document may involve patents. Distribution of this document Institutions do not assume the responsibility to identify these patents.

This standard is proposed and managed by the National Certification and Accreditation Administration Committee.

This standard was drafted. People's Republic of China Sichuan Exit Inspection and Quarantine.

1 Scope

This standard specifies the pig bacteria Lawsonia intracellular fluorescence PCR quarantine technical requirements.

This standard is applicable to the detection of porcine Lawsonia intracellular bacteria.

2 Normative references

The following documents for the application of this document is essential, dated references, only the dated version suitable for use herein Member. For undated references, the latest edition (including any amendments) applies to this document.

Laboratory use specifications and test methods GB/T 6682 Analysis SN/T 2123 Entry and Exit Animal Quarantine experimental sample collection, transport and storage specifications

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3 Instruments and reagents

3.1

The main instruments Fluorescence quantitative PCR instrument, high-speed refrigerated centrifuge, Vortex, a constant temperature circulating water bath, cold refrigerator, single head adjustable micropipette (0.1μL ~ 2.5μL, 0.5μL ~ 10.0μL, 10μL ~ 100μL, 20μL ~ 200μL).

3.2 Reagents

Qiagen DNA extraction kit animal manure 1); Qiagen animal blood and tissue DNA extraction kit 1); ABI or Bio-Rad fluorescence quantitative PCR reaction mix 1); unless otherwise specified, are to meet this standard test water GB/T 6682 tertiary water.

Sequences of primers and probes used in PCR amplification of fluorescence are shown in Table 1.

Table 1 Primers and TaqMan probes

Primer and probe sequences of length/bp Tm value/°C GC content /% Upstream primer 5'-GAGCACGATTACAAATTGCTTCA-3 '23 51.7 39 Downstream primer 5'-TGCTATCTCTGCTGCATGTAATGA-3 '24 54 42 TaqMan probe 5'-FAM-TCCCTGCACCTCCTTGAATACAATCCACA-TAMRA-3 '29 61.5 48

1) The name listed in the standard product, suitable examples of commercially available products Qiagen, ABI, or Bio-Rad company, giving this information to parties Users of this standard would not represent an endorsement of those products.

4.1 Stool sample collection and storage

Samples collected fresh faeces best use of antimicrobial drugs in animals before, collected from the rectum. When a small amount of the acquisition, sterilized cotton

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