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

SN/T 5227.9-2019

**Rapid detection of fox-derived components in exported foods
Recombinase-mediated strand replacement nucleic acid
amplification method (RAA method)
(English Translation)**

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Foreword

SN/T 5227-2019 "Rapid Detection of Animal-derived Components in Exported Foods Recombinase Mediated Strand Replacement Nucleic Acid Amplification Method (RAA Law)" is divided into 11 parts.

Part 1: Rapid detection of chicken-derived components in exported foods, recombinase-mediated strand replacement nucleic acid amplification method (RAA method);

Part 2: Rapid detection of pig-derived components in export foods, recombinase-mediated strand replacement nucleic acid amplification method (RAA method);

Part 3: Rapid detection of sheep-derived components in export foods, recombinase-mediated strand replacement nucleic acid amplification method (RAA method);

Part 4: Rapid detection of duck-derived components in export foods recombinase-mediated strand replacement nucleic acid amplification method (RAA method);

Part 5: Rapid detection of bovine-derived components in exported foods, recombinase-mediated strand replacement nucleic acid amplification method (RAA method);

Part 6: Rapid detection of buffalo-derived components in export foods recombinase-mediated strand replacement nucleic acid amplification method (RAA method);

Part 7: Rapid detection of horse-derived components in export foods, recombinase-mediated strand replacement nucleic acid amplification method (RAA method);

Part 8: Rapid detection of donkey-derived components in export foods recombinase-mediated strand replacement nucleic acid amplification method (RAA method);

Part 9: Rapid detection of fox-derived components in export foods recombinase-mediated strand replacement nucleic acid amplification method (RAA method);

Part 10: Rapid detection of mink-derived components in exported foods, recombinase-mediated strand replacement nucleic acid amplification method (RAA method);

Part 11: Rapid detection of rat-derived components in export foods Recombinase-mediated strand replacement nucleic acid amplification method (RAA method).

This part is part 9 of SN/T 5227-2019.

This part is compiled in accordance with the rules of GB/T 1.1-2009.

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

Drafting organizations of this section. Zhengzhou Customs of the People's Republic of China, Chinese Academy of Inspection and Quarantine, Zhengzhou College of Animal Husbandry Economics, Zhejiang Institute of Food and Drug Inspection, Hefei Customs of the People's Republic of China, Gongbei Customs of the People's Republic of China, People's Republic of China Xiamen Customs, Dalian Customs of the People's Republic of China, Beijing Customs of the People's Republic of China, Fuzhou Customs of the People's Republic of China, Hangzhou Public Test Biological Technology Co., Ltd.

1 Scope

This section specifies the RAA detection method for fox-derived ingredients in exported food.

This section applies to the qualitative detection of fox-derived ingredients in exported food. The minimum detection limit (LOD) of the method specified in this standard is 0.01% (W/W).

2 Normative references

The following documents are indispensable for the application of this document. For dated reference documents, only the dated version is applicable to this file. 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/T 27403-2008 Laboratory Quality Control Specification Food Molecular Biology Testing

3.1 Terms and definitions

The following terms and definitions apply to this document.

3.1.1 Vulpes the Fox

Carnivora, Schizopod, Canidae, Fox subfamily, Fox genus. Commonly known as fox, it is actually a collective name for a class of animals. There are many kinds, divided into Arctic fox, red fox, silver black fox, sand fox, etc.

3.1.2 Real-time RAA real-time RAA

A constant-temperature nucleic acid rapid amplification technology (referred to as RAA technology) that recombinase-mediated strand replacement. Utilize what is obtained from bacteria or fungi Recombinant enzyme, at a constant temperature (usually 37~42 °C), the recombinase can be tightly combined with the primer to form a polymer of the enzyme and the primer.

With the help of the DNA-binding protein, the double-stranded structure of the template DNA is opened, and when the primer is searched on the template DNA, it matches exactly with it Under the action of DNA polymerase, a new complementary DNA strand is formed, and the amplified product grows exponentially. Use fluorescence with the labeling of the probe, as the RAA reaction progresses, the RAA product shows a corresponding relationship with the increase of the fluorescence signal.

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

The time required for the fluorescence signal in each reaction tube to reach the set threshold.

3.2 Abbreviations

The following abbreviations apply to this document.

4 Method summary

Using the extracted DNA as a template, using Fox's specific detection primers and probes for real-time fluorescent RAA amplification, according to the real-time fluorescent the increase in RAA has enabled the detection and identification of fox components in food and feed.

5 Reagents and materials

Unless otherwise specified, all reagents are analytical reagents or biochemical reagents. The experimental water meets the requirements of GB/T 6682. All reagents are Dispense with DNase-free containers.

Note: See Appendix A for the target gene sequence. F is the upstream primer, R is the downstream primer, P is the probe, FAM-dT, THF, BQH-dT and C3spacer all are probe modification groups.

5.1

CTAB extraction buffer (pH8.0). 10 g/L CTAB, 0.7 mol/L NaCl, 0.05 mol/L Tris-HCl, 0.01 mol/L Na₂EDTA.

5.2

Phenol. Chloroform. Isoamyl alcohol=25.24.1.

5.3

Isopropanol.

5.4 70% ethanol (V/V).

5.5

TE buffer (pH 8.0). 10 mmol/L Tris-HCl (pH 8.0), 1 mmol/L EDTA (pH 8.0).

5.6

A Buffer. 20% polyethylene glycol.

5.7

Real-time fluorescent RAA amplification system. 2.5 mmol/L dNTPs, 225 ng/μL SSB, 300 ng/μL recA recombinase protein (SC-recA/BS-recA), 75 ng/μL Bsu DNA polymerase, 75 ng/μL Exo exonuclease, 250 mmol/L Tricine, 12.5 mmol/L dithiothreitol, 250 ng/μL creatine