

ICS 67.050
CCS C 53

SN

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

SN/T 5227.5-2019

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

(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 Terms, definitions and abbreviations
3.1 Terms and definitions
3.2 Abbreviations
4 Method summary
5 Reagents and materials
6 Equipment
7 Detection steps
7.1 DNA extraction
7.2 Determination of DNA concentration and purity
7.3 Real-time fluorescent RAA amplification
8 Quality control
8.1 Real-time fluorescent PCR instrument
8.2 Constant temperature fluorescence detector
9 Judgment and expression of results
9.1 Judgment of results

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 recombinaase-mediated strand replacement nucleic acid amplification method (RAA method);

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

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

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

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

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

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

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

This part is part 5 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, Beijing, People's Republic of China Customs, Shandong Institute of Food and Drug Inspection, Hangzhou Customs of the People's Republic of China, Gongbei Customs of the People's Republic of China, People of China Dalian Customs of the Republic of China, Xiamen Customs of the People's Republic of China, Chengdu Customs of the People's Republic of China, Fuzhou Customs of the People's Republic of China.

1 Scope

This part specifies the RAA detection method for bovine-derived components (yak and yak) in exported food.

This section applies to the qualitative detection of bovine-derived components (yak and yak) in exported food. The minimum detection of the method specified in this standard the limit (LOD) is 0.1% (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 Bovine

Artiodactyla, ruminant suborder, Bovidae, Bovis subfamily, Bovis, Boss, there are 6 species under the genus Buffalo (Indian bison), Yak, Kuwa bison (white rump bison), Cambodian bison (Khmer cow), primitive cow, bison (white-legged bison).

3.1.2 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.

3.1.3 Ct value cycle threshold

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

3.1.4 T value Time 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.

3.2.1

RAA. recombinase-aid amplification, recombinase-mediated amplification.

3.2.2

DNA. deoxyribonucleic acid, deoxyribonucleic acid.

3.2.3

CTAB. cetyltrimethylammonium bromide, cetyltrimethylammonium bromide.

3.2.4

Tris. tris (Hydroxymethyl) aminomethane.

3.2.5

EDTA. ethylene diaminetetraacetic acid.

3.2.6

dNTPs. deoxyribonucleoside triphosphate, deoxyribonucleoside triphosphate.

3.2.7

SSB. single stranded DNA binding protein, single stranded DNA binding protein.

3.2.8

SC-recA. *Streptomyces coelicolor* recombinase, *Streptomyces coelicolor* recombinase.

3.2.9

BS-recA. *Bacillus subtilis* recombinase, *Bacillus subtilis* recombinase.

3.2.10

Bsu. *Bacillus subtilis*, *Bacillus subtilis*.