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CCS X 16

SN

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

SN/T 1632.3-2013

Replacing SN/T 1632.3-2005

**Determination of Enterobacter sakazakii (Cronobacter
spp.) from dehydrated powdered milk for export. Part 3:
Real-time PCR method
(English Translation)**

Issued on: January 1, 1970

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Foreword

SN/T 1632 "Enterobacter sakazakii in milk powder exports (Craunot sp) test methods" is divided into three parts.

--- Part 1. Separation and counting;

Part --- Article 2. PCR method;

--- Part 3. Fluorescent PCR method.

This section SN/T 1632 Part 3.

In accordance with this part of GB/T 1.1-2009 given rule drafting.

The partial replacement of SN/T 1632.3-2005 "Enterobacter sakazakii in milk powder test methods - Part 3. Fluorescent PCR method." this section compared with the SN/T 1632.3-2005, in addition to editorial changes, the major technical changes are as follows.

--- Modify the standard English name;

--- Modified primers and probes;

--- Deleted the definitions, terms and abbreviations;

--- Increased Craunot genus typing method of identifying species within the informative appendix.

Please note that some of the content of this document may involve patents. Release mechanism of the present document does not assume responsibility for the identification of these patents.

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

This section drafted by. Chinese Academy of Inspection and Quarantine, Shenzhen

People's Republic of China Exit Inspection and Quarantine, the People's Republic of China Exit Inspection and Quarantine and the State of Tianjin.

1 Scope

SN/T 1632 provisions of this part of the milk powder *Enterobacter sakazakii* (Craunot sp) fluorescence PCR detection method.

This section applies to milk *Enterobacter sakazakii* (Craunot sp) the rapid qualitative detection of other foods may refer to.

2 Normative references

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

SN/T 1632.1 *Enterobacter sakazakii* in milk powder exports (Craunot sp) test methods - Part 1. Separation and counting SN/T 1632.2-2013 exports of milk powder *Enterobacter sakazakii* (Craunot sp) test methods Part 2. PCR Method

3.1 Method summary

Milk powder by enrichment, take enrichment broth added 1.5mL 1mL sterile centrifuge tubes, centrifuged at 5min 8000r/min and gently the supernatant was removed, upside down on absorbent paper, sucking the liquid, different samples should be blotted on absorbent paper in different places; the extract was added 50μLDNA (Thawed at room temperature before use and mix well, fast draw), mix boiling water bath for 5min, centrifuged 5min 12000r/min, whichever is the supernatant was used as a template for PCR amplification of fluorescence, was observed in real time PCR instrument curve, so that the milk powder *Enterobacter sakazakii* (Crowe Connaught sp) rapid test.

3.2 Reagents and materials

See SN/T 1632.1. Unless otherwise specified, reagents were analytical grade or biochemical reagents, water sterile double distilled water.

3.2.1

primer.

5'-GGCGAGCGGCGAATATTAT-3 ' 5'-CGGGTTTTCCAGTTGAGATC-3 '

3.2.2

Probe.

5'-FAM-CACCAGTTTTTCGGTGCGCCAGC-BHQ-3 '

3.2.3

ExTaqDNA polymerase.

3.2.4

dNTPs. dATP, dTTP, dCTP, dGTP.

3.2.5

DNA Extraction Reagents. 0.1% Chelex100 (chelating resins) aqueous solution.

3.2.6 10 x PCR buffer. 200mmol/L Tris-HCl (pH8.4), 200mmol/L KCl, 15mmol/L magnesium chloride.

3.2.7

FastSartUniversalProbeMaster (2 x).

3.2.8

Enterobacter sakazakii (Craunot sp) control strains. ATCC29544, or equivalent strains.

3.2.9

instruments and equipment.

3.2.10

fluorescent PCR instrument.