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Milk and milk products — Detection of *Enterobacter sakazakii*

*Lait et produits laitiers — Détection de l'*Enterobacter sakazakii

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Foreword

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ISO/TS 22964|IDF/RM 210 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

Foreword

IDF (the International Dairy Federation) is a worldwide federation of the dairy sector with a National Committee in every member country. Every National Committee has the right to be represented on the IDF Standing Committees carrying out the technical work. IDF collaborates with ISO in the development of standard methods of analysis and sampling for milk and milk products.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the Action Teams and Standing Committees are circulated to the National Committees for voting. Publication as an International Standard requires approval by at least 50 % of IDF National Committees casting a vote.

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Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. IDF shall not be held responsible for identifying any or all such patent rights.

ISO/TS 22964|IDF/RM 210 was prepared by the International Dairy Federation (IDF) and Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products.* It is being published jointly by IDF and ISO.

All work was carried out by the Joint ISO-IDF Action Team on *Harmonization*, of the Standing Committee on *Microbiological methods of analysis*, under the aegis of its project leaders, Mr D.J.C. van den Berg (NL) and Mr H. Joosten (CH).

Milk and milk products — Detection of *Enterobacter sakazakii*

1 Scope

This Technical Specification specifies a method for the detection of *Enterobacter sakazakii* in milk powder and powdered infant formula.

The method is also applicable to environmental samples collected from milk powder or infant formula factories.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 8261|IDF 122, *Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination*

ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
presumptive *Enterobacter sakazakii*
microorganisms which form typical colonies on a chromogenic isolation agar, when tests are carried out in accordance with this Technical Specification

3.2
Enterobacter sakazakii
microorganisms which form typical colonies on a chromogenic isolation agar, form yellow colonies on tryptone soya agar and display biochemical characteristics as described, when tests are carried out in accordance with this Technical Specification

4 Principle (see also annex A)

4.1 Pre-enrichment in non-selective liquid medium

The pre-enrichment medium is inoculated with the test portion and incubated at 37 °C ± 1 °C for 16 h to 20 h.

4.2 Enrichment in selective liquid medium

The selective enrichment medium is inoculated with the culture obtained in 4.1 and incubated at 44 °C ± 0,5 °C for 22 h to 26 h.

4.3 Plating out and identification

A chromogenic agar is inoculated with the enrichment culture obtained in 4.2 and incubated at 44 °C ± 1 °C for 22 h to 26 h.

4.4 Confirmation

Typical colonies are selected from the chromogenic agar, and isolates producing a yellow pigment on tryptone soya agar are biochemically characterized.

5 Culture media and reagents

5.1 General

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity. The water shall be free from substances that might inhibit the growth of microorganisms under the test conditions specified in this Technical Specification. See also ISO 6887-1 and ISO 8261|IDF 122.

In order to improve the reproducibility of the results, it is recommended that, for the preparation of culture media, dehydrated basic components or dehydrated complete media be used. In that case, follow the manufacturer's instructions rigorously. See also ISO 6887-1.

The pH values given refer to a temperature of 25 °C. Adjustments, if necessary, are made by adding either hydrochloric acid [c(HCl) = 1 mol/l] or sodium hydroxide solution [c(NaOH) = 1 mol/l].

If not used immediately, store the prepared culture media and reagents under conditions that do not produce any change in their composition, in the dark at a temperature between 0 °C and 5 °C, for no longer than 1 month, unless otherwise stated.

5.2 Culture media

5.2.1 Buffered peptone water (BPW)

5.2.1.1 Composition

Enzymatic digest of casein	10,0 g
Sodium chloride (NaCl)	5,0 g
Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12\ H_2O$)	9,0 g
Potassium dihydrogen phosphate (KH_2PO_4)	1,5 g
Water	1 000 ml

5.2.1.2 Preparation

Dissolve each of the components in the water, by heating if necessary. Adjust the pH, if necessary, to 7,0 ± 0,2 at 25 °C. Distribute the BPW in flasks or tubes according to the analytical needs. Sterilize at 121 °C for 15 min.

5.2.2 Modified lauryl sulfate tryptose broth (mLST)/vancomycin medium

5.2.2.1 Modified lauryl sulfate tryptose broth (mLST)

5.2.2.1.1 Composition

Sodium chloride (NaCl)	34,0 g
Enzymatic digest of animal and plant tissue	20,0 g
Lactose ($C_{12}H_{22}O_{11}$)	5,0 g
Potassium dihydrogen phosphate (KH_2PO_4)	2,75 g
Dipotassium hydrogen phosphate (K_2HPO_4)	2,75 g
Sodium lauryl sulfate ($C_{12}H_{25}NaO_5S$)	0,1 g
Water	1 000 ml

5.2.2.1.2 Preparation

Dissolve each of the components in the water, by heating if necessary.

Adjust the pH, if necessary, to 6,8 ± 0,2 at 25 °C. Dispense 10 ml of mLST into tubes of dimensions 18 mm × 160 mm.

Sterilize the tubes at 121 °C for 15 min.

5.2.2.2 Vancomycin solution

5.2.2.2.1 Composition

Vancomycin	10 mg
Water	10 ml

5.2.2.2.2 Preparation

Dissolve the vancomycin in the distilled water. Mix and sterilize by filtration.

The vancomycin solution may be kept at 0 °C to 5 °C for 15 days.

5.2.2.3 mLST/vancomycin medium

Add 0,1 ml of vancomycin solution (5.2.2.2.2) to 10 ml of mLST solution (5.2.2.1.2) so as to obtain a final vancomycin concentration of 10 µg per millilitre of mLST.

The complete mLST/vancomycin medium may be kept at 0 °C to 5 °C for 1 day.

5.2.3 *Enterobacter sakazakii* isolation agar (ESIA™)[1)]

5.2.3.1 Composition

Pancreatic peptone of casein	7,0 g
Yeast extract	3,0 g
Sodium choride (NaCl)	5,0 g
Sodium desoxycholate	0,6 g
5-Bromo-4-chloro-3-indolyl α-D-glucopyranoside ($C_{14}H_{15}BrClNO_6$)	0,15 g
Crystal violet	2 mg
Agar	12,0 g to18,0 g[a]
Water	1 000 ml
[a] Depending on the gel strength of the agar.	

5.2.3.2 Preparation

Dissolve each of the components in the water by boiling. Adjust the pH, if necessary, to 7,0 ± 0,2 at 25 °C. Sterilize at 121 °C for 15 min.

Cool to between 44 °C and 47 °C. Pour about 15 ml of ESIA™ medium into sterile empty Petri dishes and allow to solidify on a cool even surface.

The medium may be kept at 0 °C to 5 °C for up to 14 days.

5.2.4 Tryptone soya agar (TSA)

5.2.4.1 Composition

Enzymatic digest of casein	15,0 g
Enzymatic digest of soya	5,0 g
Sodium chloride (NaCl)	5,0 g
Agar	9,0 g to 18,0 g[a]
Water	1 000 ml
[a] Depending on the gel strength of the agar.	

5.2.4.2 Preparation

Dissolve each of the components in the water by boiling. Adjust the pH, if necessary, to 7,3 ± 0,2 at 25 °C. Sterilize at 121 °C for 15 min. Cool to between 44 °C and 47 °C. Pour about 15 ml of TSA into sterile empty Petri dishes and allow to solidify on a cool even surface.

1) ESIA™ is the trade name of a product supplied by AES Laboratoire, Rue Maryse Bastié, Ker Lann, F-35172 Bruz (FR) . This information is given for the convenience of users of this Technical Specification|IDF Reviewed Method and does not constitute an endorsement by either ISO or IDF of the product named. Equivalent products may be used if they can be shown to lead to the same results.

5.2.5 Media and reagents for biochemical characterization

5.2.5.1 Reagent for detection of oxidase

5.2.5.1.1 Composition

N,N,N′,N′-Tetramethyl-*p*-phenylenediamine dihydrochloride ($C_{10}H_{16}N_2 \cdot 2HCl$)	1,0 g
Water	100 ml

5.2.5.1.2 Preparation

Dissolve the component in the water immediately before use.

5.2.5.2 L-Lysine decarboxylation medium

5.2.5.2.1 Composition

L-Lysine monohydrochloride ($C_6H_{14}N_2O_2 \cdot HCl$)	5,0 g
Yeast extract	3,0 g
Glucose ($C_6H_{12}O_6$)	1,0 g
Bromocresol purple	0,015 g
Water	1 000 ml

5.2.5.2.2 Preparation

Dissolve each of the components in the water, by heating if necessary. Adjust the pH, if necessary, so that after sterilization it is 6,8 ± 0,2 at 25 °C. Dispense 5 ml of L-lysine decarboxylation medium into tubes of dimensions 18 mm × 160 mm.

Sterilize the tubes at 121 °C for 15 min.

5.2.5.3 L-Ornithine decarboxylation medium

5.2.5.3.1 Composition

L-Ornithine monohydrochloride ($C_5H_{12}N_2O_2 \cdot HCl$)	5,0 g
Yeast extract	3,0 g
Glucose ($C_6H_{12}O_6$)	1,0 g
Bromocresol purple	0,015 g
Water	1 000 ml

5.2.5.3.2 Preparation

Dissolve each of the components in the water, by heating if necessary. Adjust the pH, if necessary, so that after sterilization it is 6,8 ± 0,2 at 25 °C.

Dispense 5 ml of L-ornithine decarboxylation medium into tubes of dimensions 18 mm × 160 mm. Sterilize the tubes at 121 °C for 15 min.