



BSI Standards Publication

Microbiology of the food chain — Horizontal method for the detection of *Cronobacter* spp.

National foreword

This British Standard is the UK implementation of EN ISO 22964:2017.

The UK participation in its preparation was entrusted to Technical Committee AW/9, Microbiology.

A list of organizations represented on this committee can be obtained on request to its secretary.

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European foreword

This document (EN ISO 22964:2017) has been prepared by Technical Committee ISO/TC 34 "Food products" in collaboration with Technical Committee CEN/TC 275 "Food analysis - Horizontal methods" the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by October 2017, and conflicting national standards shall be withdrawn at the latest by October 2017.

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

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Endorsement notice

The text of ISO 22964:2017 has been approved by CEN as EN ISO 22964:2017 without any modification.

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

This document was prepared by the European Committee for Standardization (CEN) Technical Committee CEN/275, *Food Analysis — Horizontal methods*, in collaboration with ISO Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in accordance with the agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This first edition cancels and replaces ISO/TS 22964:2006, which has been technically revised with the following changes:

- the scope has been extended to *Cronobacter* spp. detection in food products for humans and feeding animals and environmental samples and the title changed accordingly;
- the enrichment broth, modified lauryl sulfate tryptose broth (mLST), has been replaced by *Cronobacter* selective broth (CSB);
- the isolation agar, *Enterobacter sakazakii* isolation agar (ESIA) has been replaced by chromogenic *Cronobacter* isolation (CCI) agar;
- several confirmation tests have been replaced by other tests according to [Table 1](#) of this document.

Introduction

This document describes a horizontal method for the detection of *Cronobacter* spp. in food, in animal feed and in environmental samples. The main changes, listed in the foreword, introduced in this document compared to ISO/TS 22964:2006 are considered as major (see ISO 17468[2]).

Because of the large variety of food and feed products, this horizontal method may not be appropriate in every detail for certain products. In this case, different methods, which are specific to these products, may be used if absolutely necessary for justified technical reasons. Nevertheless, every attempt should be made to apply this horizontal method as far as possible.

When this document is next reviewed, account will be taken of all information then available regarding the extent to which this horizontal method has been followed and the reasons for deviations from this method in the case of particular products.

The harmonization of test methods cannot be immediate and, for certain groups of products, International Standards and/or national standards may already exist that do not comply with this horizontal method. It is hoped that when such standards are reviewed they will be changed to comply with this document so that eventually the only remaining departures from this horizontal method will be those necessary for well-established technical reasons.

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Microbiology of the food chain — Horizontal method for the detection of *Cronobacter* spp.

1 Scope

This document specifies a horizontal method for the detection of *Cronobacter* spp.

Subject to the limitations discussed in the introduction, this document is applicable to

- food products and ingredients intended for human consumption and the feeding of animals, and
- environmental samples in the area of food production and food handling.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 6887 (all parts), *Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*

[ISO 7218](#), *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

[ISO 11133](#), *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at <http://www.electropedia.org/>
- ISO Online browsing platform: available at <http://www.iso.org/obp>

3.1

***Cronobacter* spp.**

microorganisms which form typical colonies on chromogenic *Cronobacter* isolation (CCI) agar^[10] and which display the biochemical characteristics described, when tests are carried out in accordance with this document

3.2

detection of *Cronobacter* spp.

determination of *Cronobacter* spp. (3.1) in a particular mass or volume of product or surface area when tests are carried out in accordance with this document

4 Abbreviated terms

For the purposes of this document, the following abbreviations apply.

BPW	buffered peptone water
CCI	chromogenic <i>Cronobacter</i> isolation
CSB	<i>Cronobacter</i> selective broth
TSA	tryptone soya agar

5 Principle

5.1 Non-selective pre-enrichment in BPW

A test portion is inoculated into BPW, then incubated between 34 °C and 38 °C for 18 h ± 2 h.

NOTE *Cronobacter* spp. can be present in small numbers accompanied by other *Enterobacteriaceae* such as *E. cloacae* that could interfere with their detection.

5.2 Enrichment in a selective medium (CSB)

The selective enrichment medium is inoculated with the culture obtained in [5.1](#) and incubated at 41,5 °C ± 1 °C for 24 h ± 2 h.

5.3 Plating out and identification on chromogenic agar (CCI agar)

The chromogenic agar is streaked for isolation with the enrichment culture obtained in [5.2](#) and incubated at 41,5 °C ± 1 °C for 24 h ± 2 h.

5.4 Confirmation

Typical colonies are selected from the chromogenic agar, purified on a non-selective agar such as TSA and biochemically characterized.

6 Culture media and reagents

For current laboratory practice, see [ISO 7218](#) and [ISO 11133](#).

Composition of culture media and reagents and their preparation are described in [Annex B](#).

For performance testing of culture media see [ISO 11133](#) and/or [Annex B](#).

7 Equipment and consumables

Disposable equipment is an acceptable alternative to reusable glassware if it has suitable specifications.

Usual microbiological laboratory equipment (see [ISO 7218](#)) and, in particular, the following.

7.1 Apparatus for dry sterilization (oven) or wet sterilization (autoclave).

As specified in [ISO 7218](#).

7.2 Incubators, capable of operating in the range 34 °C to 38 °C, 37 °C ± 1 °C and 41,5 °C ± 1 °C.