

Microbiology of food and animal feeding stuffs — Horizontal method for the detection of presumptive pathogenic *Yersinia enterocolitica*

The European Standard EN ISO 10273:2003 has the status of a
British Standard

ICS 07.100.30

National foreword

This British Standard is the official English language version of EN ISO 10273:2003. It is identical with ISO 10273:2003. It supersedes BS 5763-16:1995 which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee AW/9, Microbiology, which has the responsibility to:

- aid enquirers to understand the text;
- present to the responsible international/European committee any enquiries on the interpretation, or proposals for change, and keep the UK interests informed;
- monitor related international and European developments and promulgate them in the UK.

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

Cross-references

The British Standards which implement international or European publications referred to in this document may be found in the *BSI Catalogue* under the section entitled “International Standards Correspondence Index”, or by using the “Search” facility of the *BSI Electronic Catalogue* or of British Standards Online.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

Compliance with a British Standard does not of itself confer immunity from legal obligations.

Summary of pages

This document comprises a front cover, an inside front cover, the EN ISO title page, the EN ISO foreword page, the ISO title page, pages ii to v, a blank page, pages 1 to 31, the Annex ZA page, an inside back cover and a back cover.

The BSI copyright notice displayed in this document indicates when the document was last issued.

Amendments issued since publication

Amd. No.	Date	Comments
14788 Corrig. No. 1	17 October 2003	Incorporating corrected EN ISO foreword page and adding Annex ZA page

This British Standard was published under the authority of the Standards Policy and Strategy Committee on 11 July 2003

© BSI 17 October 2003

ISBN 0 580 42240 2

English version

Microbiology of food and animal feedings stuffs - Horizontal
method for the detection of presumptive pathogenic *Yersinia*
enterocolitica (ISO 10273:2003)

Microbiologie des aliments - Méthode horizontale pour la
recherche de *Yersinia enterocolitica* présumées
pathogènes (ISO 10273:2003)

Mikrobiologie von Lebensmitteln und Futtermitteln -
Horizontales Verfahren zum Nachweis von präsumtiv
pathogenen *Yersinia enterocolitica* (ISO 10273:2003)

This European Standard was approved by CEN on 20 May 2003.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Luxembourg, Malta, Netherlands, Norway, Portugal, Slovakia, Spain, Sweden, Switzerland and United Kingdom.



EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Management Centre: rue de Stassart, 36 B-1050 Brussels

CORRECTED 2003-09-24

Foreword

This document (EN ISO 10273:2003) has been prepared by Technical Committee ISO/TC 34 "Agricultural 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 December 2003, and conflicting national standards shall be withdrawn at the latest by December 2003.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Luxembourg, Malta, Netherlands, Norway, Portugal, Slovakia, Spain, Sweden, Switzerland and the United Kingdom.

Endorsement notice

The text of ISO 10273:2003 has been approved by CEN as EN ISO 10273:2003 without any modifications.

NOTE Normative references to International Standards are listed in Annex ZA (normative).

INTERNATIONAL STANDARD

**ISO
10273**

Second edition
2003-06-15

Microbiology of food and animal feeding stuffs — Horizontal method for the detection of presumptive pathogenic *Yersinia enterocolitica*

*Microbiologie des aliments — Méthode horizontale pour la recherche de
Yersinia enterocolitica présumées pathogènes*



Contents

Page

Foreword.....	iv
Introduction	v
1 Scope.....	1
2 Normative references	1
3 Terms and definitions	1
4 Principle	2
4.1 General	2
4.2 Enrichment in selective liquid media	2
4.3 Plating out and identification	2
4.4 Confirmation	2
5 Reagents and media	2
6 Apparatus and glassware.....	4
7 Sampling	5
8 Preparation of test sample	5
9 Procedure (see Annex A).....	5
9.1 Test portion and initial suspension	5
9.2 Enrichment.....	6
9.3 Plating out and identification.....	6
9.4 Confirmation	6
10 Test report.....	12
Annex A (normative) Diagram of procedure	13
Annex B (normative) Composition and preparation of culture media and reagents	14
Annex C (informative) Biochemical characteristics at 30 °C of <i>Yersinia pseudotuberculosis</i> , <i>Yersinia enterocolitica</i> and biochemically related species	29
Annex D (informative) Biovars (biotyping) of <i>Yersinia enterocolitica</i>	30
Bibliography	31

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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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

ISO 10273 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

This second edition cancels and replaces the first edition (ISO 10273:1994), Subclause 9.4 of which has been technically revised.

Introduction

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 will be made to apply this horizontal method as far as possible.

When this International Standard 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 International Standard so that eventually the only remaining departures from this horizontal method will be those necessary for well-established technical reasons.

Microbiology of food and animal feeding stuffs — Horizontal method for the detection of presumptive pathogenic *Yersinia enterocolitica*

WARNING — The use of this standard may involve hazardous materials, operations and equipment. It is the responsibility of the user of this standard to establish appropriate safety and health practices and to determine the applicability of regulatory limitations prior the use.

1 Scope

This International Standard specifies a horizontal method for the detection of *Yersinia enterocolitica* presumed to be pathogenic to human subjects. This International Standard is applicable to

- products 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 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 6887 (all parts), *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*

ISO 7218, *Microbiology of food and animal feeding stuffs — General rules for microbiological examinations*, and Amd.1:2001

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

ISO/TS 11133-1, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory*

3 Terms and definitions

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

3.1

presumptive pathogenic *Yersinia enterocolitica*

psychrotrophic bacteria forming characteristic colonies on solid selective media and having the biochemical properties meeting the pathogenicity criteria described when the test is carried out in accordance with this International Standard

3.2
detection of presumptive pathogenic *Yersinia enterocolitica*
determination of the presence or absence of these bacteria in a predetermined quantity of product, when the test is carried out in accordance with this International Standard

4 Principle

4.1 General

Presumptive pathogenic *Yersinia enterocolitica* are detected by the following three successive stages.

4.2 Enrichment in selective liquid media

The test portion is inoculated into two enrichment media

- peptone, sorbitol and bile salts (PSB) broth, and
- irgasan™, ticarcillin and potassium chlorate (ITC) broth.

The ITC broth is incubated at 25 °C for 48 h and the PSB broth for 3 to 5 days.

NOTE Enrichment in ITC broth has been proposed (see reference [1]) for the isolation of *Yersinia enterocolitica* biovar 4/serovar O:3 but not for biovar 1B serovar O:8, biovar 2/serovar O:9 (see reference [2]), or biovar 2 serovar O:5,27. Isolation of *Yersinia enterocolitica* biovar 2/serovar O:9 needs the use of an ITC medium without chlorate and which contains 80 % of the original concentration of magnesium chloride and malachite green (see reference [3]).

4.3 Plating out and identification

Using the cultures obtained in 4.2, surface plating of the following two solid selective culture media is carried out:

- agar with cefsulodin, irgasan™ and novobiocin (CIN) (see reference [7]);
- *Salmonella/Shigella* agar, with sodium desoxycholate and calcium chloride (SSDC).

The media are incubated at 30 °C, then examined after 24 h and, if necessary, after 48 h depending on the medium, to check if any characteristic colonies of *Yersinia enterocolitica* are present.

4.4 Confirmation

On plated-out colonies, tests for presumptive *Yersinia enterocolitica* are carried out, followed by biochemical confirmation tests, biotyping tests, tests to establish pathogenic criteria, and possibly serological tests.

5 Reagents and media

For current laboratory practice, see ISO 7218.

See ISO/TS 11133-1 for specific requirements about quality assurance and performance of media.

NOTE ISO/TS 11133-2 on practical guidelines on performance testing of culture media is under preparation.

In view of the large number of culture media and reagents and for the clarity of the text, their compositions are given in Annex B, which also includes details of dispensing, storage, etc.

5.1 Enrichment media

5.1.1 Peptone, sorbitol and bile salts (PSB) broth

See B.1.

5.1.2 Irgasan™, ticarcillin and potassium chlorate (ITC) broth

See B.2.

5.2 Plating out media

5.2.1 Cefsulodin, Irgasan™ and novobiocin (CIN) agar (see reference [7])

See B.3.

5.2.2 *Salmonella/Shigella* agar, with sodium desoxycholate and calcium chloride (SSDC)

See B.4.

5.2.3 Nutrient agar

See B.5.

5.3 Identification media and reagents

5.3.1 Urea indole medium

See B.6.

5.3.2 Reagent for indole detection

See B.7.

5.3.3 Kligler's agar

See B.8.

5.3.4 Reagent for detection of oxidase

See B.9.

5.3.5 Decarboxylation media

5.3.5.1 Lysine decarboxylation medium

See B.10.

5.3.5.2 Ornithine decarboxylation medium

See B.11.

5.3.6 Media for fermentation of carbohydrates (sucrose, rhamnose, trehalose and xylose)

See B.12.

5.3.7 Simmons' citrate medium

See B.13.

5.3.8 Tween™-esterase medium

See B.14.

5.3.9 Bile and aesculin agar

See B.15.

5.3.10 Casein soya agar

See B.16.

5.3.11 Casein soya agar, for detection of pyrazinamidase.

See B.17.

5.3.12 Ammonium iron(II) sulfate solution, for detection of pyrazinamidase.

See B.18.

5.3.13 Casein-soya agar, with magnesium and oxalate.

See B.19.

5.4 Saline solution

See B.20.

5.5 Potassium hydroxide in saline solution

See B.21.

5.6 Veal infusion broth

See B.22.

5.7 Sterile glycerol

See B.23.

6 Apparatus and glassware

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

Usual microbiology laboratory equipment and, in particular, the following.

6.1 Apparatus for dry sterilization (oven) or wet sterilization (autoclave)

See ISO 7218.

6.2 Incubators, capable of operating at $22\text{ °C} \pm 1\text{ °C}$, $25\text{ °C} \pm 1\text{ °C}$, $30\text{ °C} \pm 1\text{ °C}$ and $37\text{ °C} \pm 1\text{ °C}$.

6.3 Drying cabinet or oven, with ventilation by convection, capable of operating between $37\text{ °C} \pm 1\text{ °C}$ and $50\text{ °C} \pm 1\text{ °C}$.

6.4 Water baths or incubators, capable of operating between $22\text{ °C} \pm 1\text{ °C}$, $24\text{ °C} \pm 2\text{ °C}$ and $25\text{ °C} \pm 1\text{ °C}$, preferably with a suitable agitation device.