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Third edition  
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## **Microbiology of the food chain — Horizontal method for the detection of pathogenic *Yersinia enterocolitica***

*Microbiologie de la chaîne alimentaire — Méthode horizontale pour  
la recherche de Yersinia enterocolitica pathogènes*



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## Contents

	Page
Foreword .....	v
Introduction .....	vii
<b>1 Scope</b> .....	<b>1</b>
<b>2 Normative references</b> .....	<b>1</b>
<b>3 Terms and definitions</b> .....	<b>1</b>
<b>4 Abbreviated terms</b> .....	<b>2</b>
<b>5 Principle</b> .....	<b>2</b>
5.1 General .....	2
5.2 Direct plating from liquid enrichment medium .....	2
5.3 Enrichment in liquid enrichment medium and selective liquid enrichment medium .....	2
5.4 Plating out after enrichment and identification .....	2
5.5 Confirmation .....	3
<b>6 Culture media and reagents</b> .....	<b>3</b>
<b>7 Equipment and consumables</b> .....	<b>3</b>
<b>8 Sampling</b> .....	<b>3</b>
<b>9 Preparation of test sample</b> .....	<b>4</b>
<b>10 Procedure (as shown in <a href="#">Annex A</a>)</b> .....	<b>4</b>
10.1 Test portion and initial suspension .....	4
10.2 Direct plating on selective agar .....	4
10.3 Enrichment .....	5
10.4 Plating out and incubation of plates .....	5
10.4.1 Plating from PSB and ITC by KOH treatment on CIN agar .....	5
10.4.2 Plating from PSB and ITC by KOH treatment on chromogenic agar (optional) .....	5
10.5 Identification of characteristic colonies .....	5
10.6 Confirmation .....	6
10.6.1 General .....	6
10.6.2 Selection of colonies for confirmation .....	6
10.6.3 Determination of pathogenic <i>Yersinia</i> species .....	6
10.6.4 Confirmation of pathogenic <i>Y. enterocolitica</i> .....	8
10.6.5 Interpretation of confirmation tests for <i>Y. enterocolitica</i> .....	10
10.6.6 Interpretation of confirmation tests for pathogenic <i>Y. enterocolitica</i> .....	10
10.7 Biotyping of <i>Y. enterocolitica</i> (optional) .....	10
10.7.1 General .....	10
10.7.2 Fermentation of xylose .....	11
10.7.3 Tween-esterase test .....	11
10.7.4 Fermentation of salicin (optional) and trehalose .....	11
10.7.5 Indole formation .....	11
10.7.6 Interpretation of biotyping tests .....	11
<b>11 Expression of results</b> .....	<b>12</b>
<b>12 Performance characteristics of the method</b> .....	<b>12</b>
12.1 Interlaboratory study .....	12
12.2 Sensitivity .....	12
12.3 Specificity .....	12
12.4 LOD <sub>50</sub> .....	12
<b>13 Test report</b> .....	<b>12</b>
<b>14 Quality assurance</b> .....	<b>13</b>
<b>Annex A (normative) Diagrams of the procedures</b> .....	<b>14</b>

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<b>Annex B</b> (normative) <b>Composition and preparation of culture media and reagents</b> .....	<b>17</b>
<b>Annex C</b> (informative) <b>Method validation studies and performance characteristics</b> .....	<b>32</b>
<b>Annex D</b> (informative) <b>Procedure for cold enrichment</b> .....	<b>34</b>
<b>Bibliography</b> .....	<b>39</b>

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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](http://www.iso.org/directives)).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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](http://www.iso.org/iso/foreword.html).

This document was prepared by the European Committee for Standardization (CEN) Technical Committee CEN/TC 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 third edition cancels and replaces the second edition (ISO 10273:2003), which has been technically revised with the following main changes.

- In confirmation of pathogenic *Y. enterocolitica*, tests related to pathogenicity have been added or specified and relocated in the frontline. Accordingly, the word “presumptive” has been removed from the title wording (pathogenic *Y. enterocolitica*) since standard contains mandatory tests related to pathogenicity and allows separation of pathogenic and non-pathogenic *Y. enterocolitica*.
- Direct plating on cefsulodin, Irgasan<sup>TM1)</sup> and novobiocin (CIN) agar has been added.
- Incubation time for peptone, sorbitol and bile salts (PSB) enrichment broth and CIN agar has been changed.
- Inoculation and incubation time for Irgasan<sup>TM</sup>, ticarcillin and potassium chlorate (ITC) enrichment broth has been changed and specified.
- Salmonella/shigella agar with sodium desoxycholate and calcium chloride (SSDC) has been replaced by CIN agar and optional chromogenic medium.
- Inoculation of CIN agar without prior potassium hydroxide (KOH) treatment of enrichment broth has been changed to optional procedure (in parallel to mandatory KOH treatment).
- The preparation (shelf life) of KOH and ammonium iron(II) sulfate solutions has been specified.

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1) Irgasan<sup>TM</sup> is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

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- Suspect colonies from primary culture are streaked (purified) on CIN agar and (optionally) on chromogenic agar to facilitate better selection of characteristic colonies that need further confirmation. The use of stereomicroscope in identification of characteristic colonies is emphasized.
- All biochemical confirmation tests, except for pyrazinamidase test, can be replaced by real-time polymerase chain reaction (PCR) detection of *ail*-gene in accordance with ISO/TS 18867.
- Five confirmation tests (indole, trehalose, xylose, citrate, tween-esterase) have become optional. Test for salicin has been added as an optional (biotyping) test. Test for calcium requirements at 37°C has been replaced by congo red magnesium-oxalate (CR-MOX) test. Three tests (oxidase, Kligler's agar and ornithine decarboxylase) have been deleted.
- The procedure for cold-enrichment of *Y. enterocolitica* has been added as [Annex D](#);
- Performance characteristics have been added to [Annex C](#).
- Performance testing for the quality assurance of the culture media has been added to [Annex B](#) and [Annex D](#).

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## Introduction

This document specifies a horizontal method for the detection of *Yersinia enterocolitica* associated with human disease. Because of the large variety of food and feed products, this horizontal method may not be appropriate in every detail for certain products, and for some other products it may be necessary to use different methods. Nevertheless, it is hoped that in all cases every attempt will be made to apply this horizontal method as far as possible and that deviations from this will only be made if absolutely necessary for technical reasons.

The main changes, listed in the foreword, introduced in this document compared to ISO 10273:2003, are considered as major (see ISO 17468).

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 in the case of particular products.

The harmonization of test methods cannot be immediate and, for certain group 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.