

Microbiology of the food chain - Horizontal method for the detection of pathogenic *Yersinia enterocolitica* (ISO 10273:2017)

EESTI STANDARDI EESSÕNA

NATIONAL FOREWORD

See Eesti standard EVS-EN ISO 10273:2017 sisaldab Euroopa standardi EN ISO 10273:2017 ingliskeelset teksti.	This Estonian standard EVS-EN ISO 10273:2017 consists of the English text of the European standard EN ISO 10273:2017.
Standard on jõustunud sellekohase teate avaldamisega EVS Teatajas	This standard has been endorsed with a notification published in the official bulletin of the Estonian Centre for Standardisation.
Euroopa standardimisorganisatsioonid on teinud Euroopa standardi rahvuslikele liikmetele kättesaadavaks 26.04.2017.	Date of Availability of the European standard is 26.04.2017.
Standard on kättesaadav Eesti Standardikeskusest.	The standard is available from the Estonian Centre for Standardisation.

Tagasisidet standardi sisu kohta on võimalik edastada, kasutades EVS-i veebilehel asuvat tagasiside vormi või saates e-kirja meiliaadressile standardiosakond@evs.ee.

ICS 07.100.30

Standardite reprodutseerimise ja levitamise õigus kuulub Eesti Standardikeskusele

Andmete paljundamine, taastekitamine, kopeerimine, salvestamine elektroonsesse süsteemi või edastamine ükskõik millises vormis või millisel teel ilma Eesti Standardikeskuse kirjaliku loata on keelatud.

Kui Teil on küsimusi standardite autorikaitse kohta, võtke palun ühendust Eesti Standardikeskusega:
Koduleht www.evs.ee; telefon 605 5050; e-post info@evs.ee

The right to reproduce and distribute standards belongs to the Estonian Centre for Standardisation

No part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying, without a written permission from the Estonian Centre for Standardisation.

If you have any questions about copyright, please contact Estonian Centre for Standardisation:

Homepage www.evs.ee; phone +372 605 5050; e-mail info@evs.ee

English Version

Microbiology of the food chain - Horizontal method for the
detection of pathogenic *Yersinia enterocolitica* (ISO
10273:2017)

Microbiologie de la chaîne alimentaire - Méthode
horizontale pour la recherche de *Yersinia*
enterocolitica pathogènes (ISO 10273:2017)

Mikrobiologie der Lebensmittelkette - Horizontales
Verfahren zum Nachweis von pathogenen *Yersinia*
enterocolitica (ISO 10273:2017)

This European Standard was approved by CEN on 13 March 2017.

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 CEN-CENELEC 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 CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.



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

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

European foreword

This document (EN ISO 10273:2017) 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.

This document supersedes EN ISO 10273:2003.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

According to the CEN-CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Endorsement notice

The text of ISO 10273:2017 has been approved by CEN as EN ISO 10273:2017 without any modification.

Contents

	Page
Foreword.....	v
Introduction.....	vii
1 Scope.....	1
2 Normative references.....	1
3 Terms and definitions.....	1
4 Abbreviated terms.....	2
5 Principle.....	2
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
6 Culture media and reagents.....	3
7 Equipment and consumables.....	3
8 Sampling.....	3
9 Preparation of test sample.....	4
10 Procedure (as shown in Annex A).....	4
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
11 Expression of results.....	12
12 Performance characteristics of the method.....	12
12.1 Interlaboratory study.....	12
12.2 Sensitivity.....	12
12.3 Specificity.....	12
12.4 LOD ₅₀	12
13 Test report.....	12
14 Quality assurance.....	13
Annex A (normative) Diagrams of the procedures.....	14

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

This document is a preview generated by EVS

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).

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).

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.

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^{TM1)} 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 IrgasanTM, 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.

1) IrgasanTM 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.

- 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](#).

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.

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

WARNING — In order to safeguard the health of laboratory personnel, it is essential that tests for detecting pathogenic *Yersinia enterocolitica* are only undertaken in properly equipped laboratories, under the control of a skilled microbiologist, and that great care is taken in the disposal of all incubated materials. Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety aspects, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

1 Scope

This document specifies a horizontal method for the detection of *Y. enterocolitica* associated with human disease. It 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 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 11133:2014, *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

pathogenic *Yersinia enterocolitica*

psychrotrophic bacteria forming characteristic colonies on solid selective media and possessing the biochemical and molecular properties meeting the pathogenicity criteria described when confirmation tests are carried out in accordance with this document