
**Microbiology of the food chain —
Polymerase chain reaction (PCR)
for the detection of food-borne
pathogens — Detection of pathogenic
Yersinia enterocolitica and *Yersinia
pseudotuberculosis***

*Microbiologie de la chaîne alimentaire — Réaction de polymérisation
en chaîne (PCR) pour la détection de micro-organismes pathogènes
dans les aliments — Détection des Yersinia enterocolitica et Yersinia
pseudotuberculosis pathogènes*

get full document from standards.iteh.ai



Sample Document

get full document from standards.iteh.ai



COPYRIGHT PROTECTED DOCUMENT

© ISO 2015, Published in Switzerland

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

Contents

Page

Foreword	v
Introduction	vi
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Principles	1
4.1 General	1
4.2 Microbial enrichment	2
4.3 Nucleic acid extraction	2
4.4 Amplification and detection	2
4.5 Isolation	2
5 Reagents	2
5.1 General	2
5.2 Culture media	2
5.2.1 General	2
5.2.2 Diluent	2
5.2.3 Enrichment media	2
5.2.4 Selective solid medium	4
5.2.5 Potassium hydroxide in saline solution, KOH	5
5.3 Nucleic acid extraction	5
5.4 Reagents for PCR	5
5.5 Primers and probes	5
6 Apparatus and equipment	5
6.1 General	5
6.2 Equipment for sample preparation prior to enrichment	6
6.3 Equipment for microbial enrichment	6
6.4 Equipment for nucleic acid extraction	6
6.5 Equipment for real-time PCR	6
7 Sampling	6
8 Procedure	6
8.1 Sample preparation prior to enrichment	6
8.1.1 General	6
8.1.2 Preparation of the sample	6
8.2 Microbial enrichment	7
8.2.1 Pathogenic <i>Y. enterocolitica</i>	7
8.2.2 <i>Y. pseudotuberculosis</i>	7
8.2.3 Pathogenic <i>Y. enterocolitica</i> and <i>Y. pseudotuberculosis</i>	7
8.3 Isolation of colonies, optional	7
8.3.1 Pathogenic <i>Y. enterocolitica</i>	7
8.3.2 <i>Y. pseudotuberculosis</i>	7
8.3.3 Process controls	8
8.4 Nucleic acid extraction	8
8.5 PCR amplification	8
8.5.1 General	8
8.5.2 PCR controls	8
8.6 Confirmation of the PCR product	8
8.6.1 General	8
8.6.2 Interpretation of the PCR result	8
9 Test report	9

Annex A (normative) PCR detection and isolation of pathogenic *Y. enterocolitica* (see

Figure A.1)	10
Annex B (informative) Real-time PCR for detection of <i>Y. enterocolitica</i>	11
Annex C (informative) Detection and isolation of <i>Y. pseudotuberculosis</i>	21
Annex D (informative) Simultaneous detection of pathogenic <i>Y. enterocolitica</i> and <i>Y. pseudotuberculosis</i> using multiplex real-time PCR	26
Bibliography	29

Sample Document

get full document from standards.iteh.ai

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 meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: [Foreword - Supplementary information](#)

The committee responsible for this document is the European Committee for Standardization (CEN) Technical Committee CEN/TC 275, *Food analysis — Horizontal methods*, in collaboration with 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).

Introduction

Yersinia enterocolitica and *Yersinia pseudotuberculosis* are zoonotic bacterial pathogens causing food-borne infection (yersiniosis) in humans worldwide. The main reservoir for pathogenic *Y. enterocolitica* is domestic pigs[3] and for *Y. pseudotuberculosis* a wide range of domestic and wild animals such as rodents, deer, birds, and various farm animals serve as potential reservoirs.[4] Some of the biotypes of *Y. enterocolitica* are associated with human infection. In contrast, all *Y. pseudotuberculosis* are considered potentially pathogenic to humans.[9]

The chromosomally located gene *ail* (attachment invasion locus) is present in all bio(sero)types of *Y. enterocolitica* associated with disease and a variant of it is also present in *Y. pseudotuberculosis*. [8] The *ail* gene is the target gene used for detection in this Technical Specification, and the developed primer/probe sets target different sites of the *ail* gene for the two pathogens. [7][8][13][14]

Sample Document

get full document from standards.iteh.ai

Microbiology of the food chain — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — Detection of pathogenic *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*

1 Scope

This Technical Specification specifies two horizontal methods for detection of the pathogenic bioserotypes of *Y. enterocolitica* and one for detection of *Y. pseudotuberculosis* by using real-time PCR-based methods. The described methods allow for the detection of the two pathogens in enrichments and allow the isolation of colonies. *Y. pestis*, the causative agent of bubonic and pneumonic plague harbours a variant of the *ail* gene as well and will be detected by the same primer/probe set as *Y. pseudotuberculosis*. However, *Y. pestis* is normally not associated with food. This Technical Specification is applicable to products for human consumption, animal feeding stuffs, and environmental samples.

2 Normative references

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

ISO 6887-1, *Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 1: General rules for the preparation of the initial suspension and decimal dilutions*

ISO 10273, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of presumptive pathogenic Yersinia enterocolitica*

ISO 20837, *Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — Requirements for sample preparation for qualitative detection*

ISO 20838, *Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — Requirements for amplification and detection for qualitative methods*

ISO 22119, *Microbiology of food and animal feeding stuffs — Real-time polymerase chain reaction (PCR) for the detection of food-borne pathogens — General requirements and definitions*

ISO 22174, *Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection of food-borne pathogens — General requirements and definitions*

3 Terms and definitions

For the purpose of this document, the following terms and definitions given in ISO 22174 and ISO 22119 apply.

4 Principles

4.1 General

The method comprises the following consecutive steps:

- a) Microbial enrichment (4.2);

- b) Nucleic acid extraction (4.3);
- c) Amplification and detection (4.4);
- d) Isolation (4.5).

4.2 Microbial enrichment

The number of pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* bacterial cells is increased by growth in a non-selective or semi-selective liquid nutrient medium.

4.3 Nucleic acid extraction

Bacteria cells are separated from the nutrient broth, lysed, and the nucleic acid extracted for use in the PCR reaction.

4.4 Amplification and detection

The extracted nucleic acid is amplified using a probe-based real-time PCR. Detection of the target sequence is achieved by monitoring a clear increase in the fluorescence signal above the cycle threshold, *C_t*.

NOTE Probe-based real-time PCR combines amplification, detection, and confirmation of the target DNA.

4.5 Isolation

After a PCR-positive result is obtained, the target organism can be isolated by using culture methods as described in this Technical Specification.

5 Reagents

5.1 General

For the stages in 4.1 b)-c), molecular grade reagents and consumables suitable for molecular biology shall be used as given in ISO 20837 and ISO 20838.

Requirements are specified in ISO 20838.

The following media and reagents should be used.

5.2 Culture media

5.2.1 General

See ISO 7218 and ISO 11133 for the preparation, production, and performance testing of culture media.

5.2.2 Diluent

See ISO 6887-1 and the relevant part of ISO 6887 dealing with the product to be examined.

5.2.3 Enrichment media

5.2.3.1 Tryptone-soya broth supplemented with yeast, TSBY

5.2.3.1.1 Composition

Pancreatic digest of casein	17,0 g
-----------------------------	--------

Papaic digest of soyabean meal	3,0 g
Sodium chloride, (NaCl)	5,0 g
Dibasic potassium phosphate, (K ₂ HPO ₄)	2,0 g
Glucose	2,5 g
Yeast extract	6,0 g
Water	1 000 ml

5.2.3.1.2 Preparation

Dissolve the above ingredients in 1 000 ml distilled water. Adjust the pH, if necessary, so that after sterilization it is pH 7,3 ± 0,2. Dispense the medium into tubes or flasks of suitable capacity to obtain portions appropriate for the test samples. Sterilize for 15 min at 121 °C ± 1 °C.

Store the medium in the dark at room temperature and not longer than 4 weeks.

Alternatively, use dehydrated Tryptone soya broth (TSB) 30 g/l supplemented with 0,6 % yeast extract, pH 7,3 ± 0,2.

5.2.3.2 Peptone-sorbitol-bile-salt broth, PSB^[15]

5.2.3.2.1 Composition

Peptone	5,0 g
Sorbitol	10,0 g
Sodium chloride, (NaCl)	5,0 g
Disodium hydrogen phosphate (Na ₂ HPO ₄)	8,23 g
Sodium dihydrogen phosphate monohydrate (NaH ₂ PO ₄ ·H ₂ O)	1,2 g
Bile salts	1,5 g
Water	1 000 ml

5.2.3.2.2 Preparation

Dissolve the components or the dehydrated complete medium in the water, if necessary by heating.

Adjust the pH, if necessary, so that after sterilization it is pH 7,6 ± 0,2.

Dispense the medium into tubes or flasks of suitable capacity to obtain portions appropriate for the test samples. Sterilize for 15 min at 121 °C ± 1 °C.

5.2.3.3 Cold enrichment broth, PMB ^[5]

5.2.3.3.1 Composition

Disodium hydrogen phosphate (Na ₂ HPO ₄)	7,6 g
Potassium dihydrogen phosphate (KH ₂ PO ₄)	1,0 g
Sodium chloride (NaCl)	8,5 g