

English Version

Microbiology of the food chain - Polymerase chain reaction
(PCR) for the detection of food-borne pathogens -
Detection of pathogenic *Yersinia enterocolitica* and
Yersinia pseudotuberculosis (ISO/TS 18867:2015)

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 (ISO/TS 18867:2015)

Mikrobiologie der Lebensmittelkette - Polymerase-
Kettenreaktion (PCR) zum Nachweis von pathogenen
Mikroorganismen in Lebensmitteln - Nachweis von
pathogenen *Yersinia enterocolitica* und *Yersinia*
pseudotuberculosis (ISO/TS 18867:2015)

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European foreword

This document (CEN ISO/TS 18867:2015) 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.

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The text of ISO/TS 18867:2015 has been approved by CEN as CEN ISO/TS 18867:2015 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.

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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]

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);