

2017-07-17

Mikrobiologisk undersøgelse af fødevarer og foderstoffer – Horizontal metode til påvisning af potentiel enteropatogen *Vibrio* spp. – Del 1: Påvisning af *Vibrio parahaemolyticus* og *Vibrio cholerae*

Microbiology of food and animal feeding stuffs – Horizontal method for the detection of potentially enteropathogenic *Vibrio* spp. – Part 1: Detection of *Vibrio parahaemolyticus* and *Vibrio cholerae* (ISO 21872-1:2017)



DANSK STANDARD
Danish Standards Association

Göteborg Plads 1
DK-2150 Nordhavn
Tel: +45 39 96 61 01
Fax: +45 39 96 61 02
dansk.standard@ds.dk
www.ds.dk

This is a preview of "DS/EN ISO 21872-1:20...". Click here to purchase the full version from the ANSI store.

DS projekt: 1500750
ICS: 07.100.30

Første del af denne publikations betegnelse er:

DS/EN ISO, hvilket betyder, at det er en international standard, der har status både som europæisk og dansk standard.

Denne publikations overensstemmelse er:

IDT med: ISO 21872:2017.

IDT med: EN ISO 21872-1:2017.

DS-publikationen er på engelsk.

Denne publikation erstatter: DS/ISO/TS 21872-1:2007, DS/ISO/TS 21872-1/Corr. 1:2008, DS/ISO/TS 21872-2:2007.

DS-publikationstyper

Dansk Standard udgiver forskellige publikationstyper.

Typen på denne publikation fremgår af forsiden.

Der kan være tale om:

Dansk standard

- standard, der er udarbejdet på nationalt niveau, eller som er baseret på et andet lands nationale standard, eller
- standard, der er udarbejdet på internationalt og/eller europæisk niveau, og som har fået status som dansk standard

DS-information

- publikation, der er udarbejdet på nationalt niveau, og som ikke har opnået status som standard, eller
- publikation, der er udarbejdet på internationalt og/eller europæisk niveau, og som ikke har fået status som standard, fx en teknisk rapport, eller
- europæisk præstandard

DS-håndbog

- samling af standarder, eventuelt suppleret med informativt materiale

DS-hæfte

- publikation med informativt materiale

Til disse publikationstyper kan endvidere udgives

- tillæg og rettelsesblade

DS-publikationsform

Publikationstyperne udgives i forskellig form som henholdsvis

- fuldtekstpublikation (publikationen er trykt i sin helhed)
- godkendelsesblad (publikationen leveres i kopi med et trykt DS-omslag)
- elektronisk (publikationen leveres på et elektronisk medie)

DS-betegnelse

Alle DS-publikationers betegnelse begynder med DS efterfulgt af et eller flere præfikser og et nr., fx **DS 383**, **DS/EN 5414** osv. Hvis der efter nr. er angivet et **A** eller **Cor**, betyder det, enten at det er et **tillæg** eller et **rettelsesblad** til hovedstandard, eller at det er indført i hovedstandard.

DS-betegnelse angives på forsiden.

Overensstemmelse med anden publikation:

Overensstemmelse kan enten være IDT, EQV, NEQ eller MOD

- **IDT:** Når publikationen er identisk med en given publikation.
- **EQV:** Når publikationen teknisk er i overensstemmelse med en given publikation, men præsentationen er ændret.
- **NEQ:** Når publikationen teknisk eller præsentationsmæssigt ikke er i overensstemmelse med en given standard, men udarbejdet på baggrund af denne.
- **MOD:** Når publikationen er modificeret i forhold til en given publikation.

EUROPÄISCHE NORM

July 2017

ICS 07.100.30

English Version

Microbiology of food and animal feeding stuffs - Horizontal
method for the detection of potentially enteropathogenic
Vibrio spp. - Part 1: Detection of *Vibrio parahaemolyticus*
and *Vibrio cholerae* (ISO 21872-1:2017)

Microbiologie des aliments - Méthode horizontale pour
la recherche des *Vibrio* spp. potentiellement
entéropathogènes - Partie 1: Recherche de *Vibrio*
parahaemolyticus et *Vibrio cholerae* (ISO 21872-
1:2017)

Mikrobiologie von Lebensmitteln und Futtermitteln -
Horizontales Verfahren zum Nachweis von potentiell
enteropathogenen *Vibrio* spp. - Teil 1: Nachweis von
vibrio parahaemolyticus und *vibrio cholerae* (ISO
21872-1:2017)

This European Standard was approved by CEN on 14 May 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

This is a preview of "DS/EN ISO 21872-1:20...". [Click here to purchase the full version from the ANSI store.](#)

Contents	Page
European foreword.....	3

This is a preview of "DS/EN ISO 21872-1:20...". [Click here to purchase the full version from the ANSI store.](#)

European foreword

This document (EN ISO 21872-1:2017) 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 January 2018 and conflicting national standards shall be withdrawn at the latest by January 2018.

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

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 21872-1:2017 has been approved by CEN as EN ISO 21872-1:2017 without any modification.

This is a preview of "DS/EN ISO 21872-1:20...". [Click here to purchase the full version from the ANSI store.](#)

First edition
2017-06

**Microbiology of the food chain —
Horizontal method for the
determination of *Vibrio* spp. —**

Part 1:
**Detection of potentially
enteropathogenic *Vibrio*
parahaemolyticus, *Vibrio cholerae* and
*Vibrio vulnificus***

*Microbiologie de la chaîne alimentaire — Méthode horizontale pour
la détermination des *Vibrio* spp. —*

*Partie 1: Recherche des espèces de *Vibrio* parahaemolyticus, *Vibrio*
cholerae et *Vibrio* vulnificus potentiellement entéropathogènes*



Reference number
ISO 21872-1:2017(E)

© ISO 2017



COPYRIGHT PROTECTED DOCUMENT

© ISO 2017, 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

This is a preview of "DS/EN ISO 21872-1:20...". Click here to purchase the full version from the ANSI store.

Contents

	Page
Foreword	v
Introduction	vi
1 Scope	1
2 Normative references	1
3 Terms and definitions	2
4 Principle	2
4.1 General.....	2
4.2 Primary enrichment in a liquid selective medium.....	2
4.3 Secondary enrichment in a liquid selective medium.....	3
4.4 Isolation and identification.....	3
4.5 Confirmation.....	3
5 Culture media and reagents	3
5.1 Enrichment medium: alkaline saline peptone water (ASPW).....	4
5.2 Solid selective isolation media.....	4
5.2.1 First medium: thiosulphate, citrate, bile and sucrose agar medium (TCBS).....	4
5.2.2 Second medium.....	4
5.3 Saline nutrient agar (SNA).....	4
5.4 Reagent for detection of oxidase.....	4
5.5 Biochemical tests.....	4
5.5.1 L-lysine decarboxylase saline medium (LDC).....	4
5.5.2 Arginine dihydrolase saline medium (ADH).....	4
5.5.3 Reagent for detection of β -galactosidase.....	5
5.5.4 Saline medium for detection of indole.....	5
5.5.5 Saline peptone waters.....	5
5.5.6 Sodium chloride solution.....	5
5.6 PCR.....	5
5.6.1 Tris acetate EDTA buffer (TAE) (or a buffer allowing similar performance for the purpose).....	5
5.6.2 Mastermix.....	5
5.6.3 Primers and probes.....	5
5.6.4 Positive control material.....	5
5.6.5 Negative extraction control.....	6
6 Equipment and consumables	6
7 Sampling	6
8 Preparation of the test sample	6
9 Procedure (See Figure A.1)	7
9.1 Test portion and initial suspension.....	7
9.2 Primary selective enrichment.....	7
9.3 Secondary selective enrichment.....	7
9.4 Isolation and identification.....	8
9.5 Confirmation.....	8
9.5.1 General.....	8
9.5.2 Selection of colonies for confirmation and preparation of pure cultures.....	9
9.5.3 Tests for presumptive identification.....	9
9.5.4 Biochemical confirmation.....	10
9.5.5 PCR confirmation.....	12
9.5.6 DNA extraction.....	12
9.5.7 Conventional PCR.....	12
9.5.8 Real-time PCR.....	13
10 Expression of results	13

This is a preview of "DS/EN ISO 21872-1:20...". Click here to purchase the full version from the ANSI store.

11	Performance characteristics of the method	13
11.1	Interlaboratory study.....	13
11.2	Sensitivity.....	14
11.3	Specificity.....	14
11.4	LOD ₅₀	14
12	Test report	14
Annex A	(normative) Diagram of procedure	15
Annex B	(normative) Composition and preparation of the culture media and reagents	17
Annex C	(informative) Conventional PCR for the detection of <i>Vibrio parahaemolyticus</i>, thermostable direct haemolysin (<i>tdh</i>) and thermostable direct related haemolysin (<i>trh</i>) genes, <i>Vibrio cholerae</i> and <i>Vibrio vulnificus</i>	23
Annex D	(informative) Real-time PCR for the detection of <i>Vibrio parahaemolyticus</i>, thermostable direct haemolysin gene (<i>tdh</i>) and <i>Vibrio vulnificus</i>	27
Annex E	(informative) Results of an interlaboratory study	29
Bibliography	32

This is a preview of "DS/EN ISO 21872-1:20...". Click here to purchase the full version from the ANSI store.

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 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 TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in accordance with the agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This first edition cancels and replaces ISO/TS 21872-1:2007, which has been technically revised. It also incorporates ISO/TS 21872-1:2007/Cor.1:2008.

The main changes are as follows:

- introduction of optional molecular identification methods for major food borne *Vibrio* spp. (*V. parahaemolyticus*, including potentially enteropathogenic strains, *V. vulnificus* and *V. cholerae*);
- performance characteristics of the method have been added in [Annex E](#).

A list of all parts in the ISO 21872 series can be found on the ISO website.

Introduction

Because of the large variety of food and feed products, the horizontal method described in this document 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.

The main changes, listed in the foreword, introduced in this document compared to ISO/TS 21872-1:2007 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 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 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 determination of *Vibrio* spp. —

Part 1:

Detection of potentially enteropathogenic *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus*

WARNING — In order to safeguard the health of laboratory personnel, it is essential that tests for detection of *Vibrio* spp., and particularly toxigenic *Vibrio cholerae*, be conducted only in laboratories equipped for this purpose and under the supervision of an experienced microbiologist, and that great care is exercised in the disposal of contaminated material.

1 Scope

This document specifies a horizontal method for the detection of enteropathogenic *Vibrio* spp., which causes human illness in or via the intestinal tract. The species detectable by the methods specified include *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus*.

It is applicable to the following:

- products intended for human consumption and the feeding of animals;
- environmental samples in the area of food production and food handling.

NOTE 1 This method may not be appropriate in every detail for certain products (see Introduction).

NOTE 2 The World Health Organization (WHO) has identified that *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus* are the major food-borne *Vibrio* spp. However, the method in this document can also be appropriate for the identification of other *Vibrio* spp. causing illness in humans.^[1]

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 6887-1:2017, *Microbiology of the food chain — 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 6887-3, *Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 3: Specific rules for the preparation of fish and fishery products*

ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

ISO 11133, *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*

ISO 22118, *Microbiology of food and animal feeding stuffs — Polymerase chain reaction (PCR) for the detection and quantification of food-borne pathogens — Performance characteristics*

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 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
potentially enteropathogenic *Vibrio* spp.
microorganism which forms typical colonies on solid selective media and which possesses the described biochemical or molecular characteristics when the test is performed in accordance with this document

Note 1 to entry: This document describes specific procedures for *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus*.

3.2
detection of potentially enteropathogenic *Vibrio* spp.
determination of the presence or absence of potentially enteropathogenic *Vibrio* spp. (3.1) (*V. parahaemolyticus*, *V. cholerae* and *V. vulnificus*) in a determined quantity of product, when the test is performed in accordance with this document

4 Principle

4.1 General

The detection of potentially enteropathogenic *Vibrio* spp. (*V. parahaemolyticus*, *V. cholerae* and *V. vulnificus*) requires four successive phases, as shown in the procedure diagram in [Annex A](#).

Recovery of certain *Vibrio* spp. from foodstuffs may be improved by the use of different incubation temperatures depending upon the target species or state of the food matrix. For example, recovery of *V. parahaemolyticus* and *V. cholerae* in fresh products is enhanced by enrichment at 41,5 °C whereas for *V. vulnificus*, and for *V. parahaemolyticus* and *V. cholerae* in deep frozen (<-18 °C),^[2] dried or salted products, recovery is enhanced by enrichment at 37 °C. If detection of *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus* is required, all specified incubation temperatures should be used. If detection of *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus* together is not required, the specific procedure(s) may be selected according to the species being sought. Such a selection should be clearly specified in the test report.

NOTE *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus* may be present in small numbers and are often accompanied by a much larger number of other microorganisms belonging to the *Vibrionaceae* family or to other families.

4.2 Primary enrichment in a liquid selective medium

Inoculation of the test portion in the primary enrichment medium alkaline saline peptone water (ASPW) (5.1) at ambient temperature, followed by incubation at 41,5 °C for 6 h and/or 37 °C for 6 h.

The incubation conditions are determined by the target species and food product state.

For detection of all target species in deep frozen, dried or salted products, primary enrichment should be at 37 °C.