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Mikrobiologija v prehranski verigi - Horizontalna metoda za ugotavljanje potencialno enteropatogene Vibrio parahaemolyticus, Vibrio cholerae in Vibrio vulnificus (ISO/DIS 21872:2016)

Microbiology of the food chain - Horizontal method for the detection of potentially enteropathogenic Vibrio parahaemolyticus, Vibrio cholerae and Vibrio vulnificus (ISO/DIS 21872:2016)

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Mikrobiologie von Lebensmitteln und Futtermitteln - Horizontales Verfahren zum Nachweis von potentiell enteropathogenen Vibrio spp. - Teil 1: Nachweis von vibrio parahaemolyticus und vibrio cholerae

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Microbiologie de la chaîne alimentaire - Méthode horizontale pour la recherche des Vibrio spp. potentiellement entéropathogènes - Recherche des espèces de Vibrio parahaemolyticus, Vibrio cholerae et Vibrio vulnificu (ISO/DIS 21872:2016)

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Microbiology of the food chain — Horizontal method for the detection of potentially enteropathogenic *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus*

Microbiologie de la chaîne alimentaire — Méthode horizontale pour la recherche des Vibrio spp. potentiellement entéropathogènes — Recherche des espèces de Vibrio parahaemolyticus, Vibrio cholerae *et* Vibrio vulnificu

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This draft is hereby submitted to the ISO member bodies and to the CEN member bodies for a parallel three month enquiry.

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 21872 was prepared by 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).

This first edition cancels and replaces the technical specifications ISO/TS 21872-1:2007 and ISO/TS 21872-2:20027which have been technically revised.

Main changes :

- same procedure for the detection of major food borne *Vibrio* spp.
- performance characteristics of the method have been added to Annex E.

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Introduction

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

When this International Standard 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 harmonisation 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 International Standard so that eventually the only remaining departures from this horizontal method will be those necessary for well-established technical reasons.

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Microbiology of the food chain — Horizontal method for the detection of enteropathogenic *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus*

1 Scope

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

Taking into account the remarks made in the introduction, this International Standard is applicable to:

— products intended for human consumption and the feeding of animals;

— environmental samples in the area of food production and food handling.

NOTE 1 Reasons for not applying this method are discussed in the Introduction.

NOTE 2 The World Health Organisation (WHO) have identified that *V. parahaemolyticus, V. cholerae* and *V. vulnificus* are the major food borne *Vibrio* spp. however this standard can also be appropriate for the identification of other *Vibrio* spp. causing illness in humans [1].

WARNING — In order to safeguard the health of laboratory personnel, it is essential that tests for detection of *Vibrio*, and particularly toxigenic *V. 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.

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2 Normatives 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, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination*.

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

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

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

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.*

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3 Terms and definitions

3.1 Potentially enteropathogenic *Vibrio* spp.

microorganisms which form typical colonies on solid selective media and which possess the described biochemical or molecular characteristics when the test is performed according to this International Standard.

Note 1 to entry: This International Standard 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. *(V. parahaemolyticus, V. cholerae* and *V. vulnificus)* (3.1), in a determined quantity of product, when the test is performed in accordance with this International Standard

4 Principle

4.1 General

The detection of potentially enteropathogenic *Vibrio* spp. *(V. parahaemolyticus, V. cholerae* and *V. vulnificus)* requires four successive phases (see also 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, dried or salted products, recovery is enhanced by enrichment at 37 °C. The method combines choleragenic and specific halophilic culture-based methods for use where mixtures of *Vibrio* species are present. Users of this International Standard requiring detection of *V. parahaemolyticus, V. cholerae* and *V. vulnificus* should use all specified incubation temperatures. Users of this International Standard who do not require detection of *V. parahaemolyticus, V. cholerae* and *V. vulnificus* together may select the specific procedure(s) according to the species they are required to detect. 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. Consequently, two successive selective enrichments can be necessary in order to detect the target organisms.

4.2 Primary enrichment in a liquid selective medium

Inoculation of the test portion in the primary enrichment medium (ASPW) (5.1) at ambient temperature, followed by incubation at 41,5 °C ± 1°C for 6 h ± 1 h and 37 °C ± 1°C for 6 h ± 1 h.

For deep frozen, dried or salted products primary enrichment at 41,5 °C shall be omitted irrespective of the target species.

For detection of *V. vulnificus* only, primary enrichment at 41,5 °C shall be omitted.

For detection of *V. parahaemolyticus* and/or *V. cholerae* only, in fresh products, primary enrichment at 37 °C can be omitted.

4.3 Secondary enrichment in a liquid selective medium

Inoculation of the second enrichment medium (ASPW) with the cultures obtained in 4.2.

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Incubation of inoculated enrichment medium at 41,5 °C ± 1 °C for 18 h ± 1 h and/or 37 °C ± 1 °C for 18 h ± 1 h.

For detection of *V. parahaemolyticus* and/or *V. cholerae* only, secondary enrichment at 37 °C can be omitted.

4.4 Isolation and identification

From the cultures obtained in 4.2 and in 4.3, inoculation of two solid selective media:

— Thiosulfate Citrate Bile and Sucrose agar (TCBS) medium (5.2.1);

— another appropriate solid selective medium (left to the choice of the laboratory), such as chromogenic agar, complementary to the TCBS medium (5.2.2).

Incubation of the TCBS medium at 37 °C ± 1 °C, then examination after 24 h ± 3 h. Incubation the second selective medium according to the manufacturer's recommendations.

4.5 Confirmation

Presumptive colonies of *V. parahaemolyticus, V. cholerae* and *V. vulnificus* isolated in 4.4 are subcultured and confirmed by means of appropriate biochemical and/or polymerase chain (PCR) reaction tests.

Users of this International Standard may use biochemical and/or PCR confirmation of isolates. Detection of enteropathogenic *V. parahaemolyticus* as determined by presence of the direct thermostable haemolysin (*tdh*) and/or direct related haemolysin (*trh*) genes can only be carried out using PCR tests.

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NOTE PCR based identification can be achieved by conventional PCR for *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* or real-time PCR for *V. parahaemolyticus*, and *V. vulnificus*. The PCR methods used in the development of this Standard are given in Annexes C and D.

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5 Culture media and reagents

For general laboratory practice, see ISO 7218.

NOTE 1 On account of the large number of culture media and reagents, it has been considered preferable, for clarity of the text, to give their composition and their preparation in Annex B.

NOTE 2 Primers, probes and PCR running conditions used in the development of this Standard are given in Annexes C and D.

5.1 Enrichment medium: alkaline saline peptone water (ASPW)

See B.1.

5.2 Solid selective isolation media

5.2.1 First medium: Thiosulphate, Citrate, Bile and Sucrose agar medium (TCBS)

See B.2.

See ISO 11133.

Table 1 — Performance testing of Thiosulphate, Citrate, Bile and Sucrose agar medium (TCBS)

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Function	Incubation	Control strains	WCDM [a]	Criteria	Characteristic reactions
Productivity	37 °C ± 1 °C for 18 - 24 h	*Vibrio parahaemolyticus*	00185 [b]	Good growth	Green (sucrose negative)
	37 °C ± 1 °C for 18 - 24 h	*Vibrio furnissii*	00186 [b]	Good growth	Yellow (sucrose positive)
Selectivity	37 °C ± 1 °C for 18 - 24 h	*Escherichia coli* [c, d]	00012, 00013 or 00090	Total inhibition	-

[a] World Data Centre for Microorganisms (WDCM) strain catalogue available on http://refs.wdcm.org

[b] Strain to be used as a minimum

[c] Some national restrictions and directions may require the use of a different *E. coli* serovar. Make reference to national requirements relating to the choice of *E. coli* serovars.

[d] Strain free of choice; one of the strains has to be used as a minimum.

5.2.2 Second medium

The selection of the second medium is left to the choice of the test laboratory. Preparation of the medium should be strictly according to the manufacturer's instructions.

5.3 Saline nutrient agar (SNA)

See B.3.

5.4 Reagent for detection of oxidase

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See B.4.

5.5 Biochemical tests

5.5.1 Lysine decarboxylase saline medium (LDC)

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See B.5.

5.5.2 Arginine dihydrolase saline medium (ADH)

See B.6.

5.5.3 Reagent for detection of β-galactosidase

See B.7.

5.5.4 Saline medium for detection of indole

See B.8.

5.5.5 Saline peptone waters

See B.9.

5.5.6 Sodium chloride solution

See B.10.

5.6 PCR

5.6.1 TAE buffer (or a buffer allowing similar performance for the purpose).

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See B.11.

5.6.2 Mastermix

Reagents shall be added in quantities as specified by the manufacturer's instructions. See Annexes C and D (informative) for example details of master mixes used in the development of this Standard.

5.6.3 Primers and probes

Primer (and hydrolysis probe) sequences if required shall be published in a peer-reviewed journal and be verified for use against a broad range of target *Vibrio* and non-target strains.

For *V. parahaemolyticus* the target region should be *toxR*.

For determination of pathogen strains of *V. parahaemolyticus* genes encoding the thermostable direct (TDH) and the thermostable direct related (TRH) haemolysins should be targeted.

For *V. cholerae* the target region for conventional PCR should be the 16S-23S rRNA intergenic spacer region prVC.

For *V. vulnificus* the target region should be the cytotoxin-haemolsyin region.

Target regions other than those specified above for the identification of *V. parahaemolyticus, V. vulnificus* and *V. cholerae* can be used if they have been shown to demonstrate equivalent performance, are published in a peer-reviewed journal and are verified against a broad range of target *Vibrio* and non-target strains.

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See Annex C (informative) for example details of primers used for conventional PCR and Annex D (informative) for primers and hydrolysis probes for real-time PCR used in the development of this Standard.

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5.6.4 Positive control material

Separate control material shall be used for each target *Vibrio* spp. See Annex C and D (informative) for example details of control strains used in the development of this Standard.

5.6.5 Negative extraction control

Nuclease free water or sterile NaCl 0,85 % extracted according to 9.5.6.

6 Apparatus and glassware

Disposable equipment is acceptable in the same way as reuseable glassware, if the specifications are similar.

Ordinary microbiology laboratory equipment (ISO 7218), and in particular the following:

6.1 Refrigerator, adjustable to 5 °C ± 2,0 °C.

6.2 Incubator, adjustable to 37 °C ± 1,0 °C.

6.3 Incubator, adjustable to 41,5 °C ± 1,0 °C.

6.4 Freezer, adjustable to < -15 °C.

6.5 Micro-centrifuge tubes, with capacities of 1,5 ml and 2,0 ml.

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