

Microbiology of food and animal feeding stuffs — Horizontal method for the detection of potentially enteropathogenic *Vibrio* spp. —

Part 2: Detection of species other than
Vibrio parahaemolyticus and *Vibrio*
cholerae

ICS 07.100.30

National foreword

This Draft for Development is the UK implementation of ISO/TS 21872-2:2007.

This publication is not to be regarded as a British Standard.

It is being issued in the Draft for Development series of publications and is of a provisional nature. It should be applied on this provisional basis, so that information and experience of its practical application can be obtained.

Comments arising from the use of this Draft for Development are requested so that UK experience can be reported to the international organization responsible for its conversion to an international standard. A review of this publication will be initiated not later than 3 years after its publication by the international organization so that a decision can be taken on its status. Notification of the start of the review period will be made in an announcement in the appropriate issue of *Update Standards*.

According to the replies received by the end of the review period, the responsible BSI Committee will decide whether to support the conversion into an international Standard, to extend the life of the Technical Specification or to withdraw it. Comments should be sent to the Secretary of the responsible BSI Technical Committee at British Standards House, 389 Chiswick High Road, London W4 4AL.

The UK participation in its preparation was entrusted to Technical Committee AW/9, Microbiology.

A list of organizations represented on this committee can be obtained on request to its secretary.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

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Microbiology of food and animal feeding stuffs — Horizontal method for the detection of potentially enteropathogenic *Vibrio* spp. —

Part 2:

Detection of species other than *Vibrio* *parahaemolyticus* and *Vibrio cholerae*

*Microbiologie des aliments — Méthode horizontale pour la recherche
des Vibrio spp. potentiellement entéropathogènes —*

*Partie 2: Recherche des espèces autres que Vibrio parahaemolyticus et
Vibrio cholerae*



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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.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of normative document:

- an ISO Publicly Available Specification (ISO/PAS) represents an agreement between technical experts in an ISO working group and is accepted for publication if it is approved by more than 50 % of the members of the parent committee casting a vote;
- an ISO Technical Specification (ISO/TS) represents an agreement between the members of a technical committee and is accepted for publication if it is approved by 2/3 of the members of the committee casting a vote.

An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

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/TS 21872-2 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

ISO/TS 21872 consists of the following parts, under the general title *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*
- *Part 2: Detection of species other than Vibrio parahaemolyticus and Vibrio cholerae*

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 Technical Specification 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 Technical Specification so that eventually the only remaining departures from this horizontal method will be those necessary for well-established technical reasons.

Microbiology of food and animal feeding stuffs — Horizontal method for the detection of potentially enteropathogenic *Vibrio* spp. —

Part 2:

Detection of species other than *Vibrio parahaemolyticus* and *Vibrio cholerae*

WARNING — In order to safeguard the health of laboratory personnel, it is essential that tests for detection of *Vibrio* spp., and the 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 be exercised in the disposal of contaminated material.

1 Scope

This part of ISO/TS 21872 specifies a horizontal method for detection of the enteropathogenic *Vibrio* species, causing illness in or via the intestinal tract, other than *Vibrio parahaemolyticus* and *Vibrio cholerae*. The species detectable by the methods specified include *Vibrio fluvialis*, *Vibrio mimicus* and *Vibrio vulnificus*¹⁾. It is not suitable for the isolation of *Vibrio hollisae*. Strains of *V. parahaemolyticus* and *V. cholerae* may also be detected during the application of this method.

This part of ISO/TS 21872 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.

This method is not appropriate for the detection of *Vibrio metschnikovii* as this is oxidase negative.

NOTE 1 *Vibrio metschnikovii* has been occasionally isolated from human faecal samples and can be a cause of diarrhoeal diseases.

NOTE 2 The identification of *Vibrio* species other than *V. parahaemolyticus* and *V. cholerae* is difficult, and needs further development. The biochemical tests given in this part of ISO/TS 21872 enable only a presumptive confirmation of these species.

NOTE 3 Reasons for not applying this method are discussed in the Introduction.

2 Normative references

The following referenced documents are indispensable for the application 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.

1) See 9.4.4.

ISO 6887 (all parts), *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 requirements and guidance for microbiological examinations*

ISO 8261, *Milk and milk products — General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
potentially enteropathogenic *Vibrio*
microorganisms which form typical colonies on solid selective media and which possess the described biochemical characteristics when the test is performed in accordance with this part of ISO/TS 21872

3.2
detection of potentially enteropathogenic *Vibrio*
determination of the presence or absence of presumptive, enteropathogenic *Vibrio*, in a specified quantity of product, when the test is performed in accordance with this part of ISO/TS 21872

4 Principle

4.1 General

The detection of potentially enteropathogenic *Vibrio* spp. requires four successive phases (see also Annex A).

NOTE *Vibrio* can, indeed, 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 are necessary.

4.2 First enrichment in a liquid selective medium

The enrichment medium (alkaline saline peptone water, ASPW) (5.1) is inoculated with the test portion at ambient temperature. It is incubated at 37 °C for 6 h ± 1 h.

In the case of large quantities, the ASPW should be warmed to 37 °C before inoculation with the test portion.

4.3 Second enrichment in a liquid selective medium

The enrichment medium (ASPW) is then inoculated with the culture obtained in 4.2.

It is incubated at 37 °C for 18 h ± 1 h.

4.4 Isolation and identification

The following two solid selective media are inoculated with the cultures obtained in 4.2 and in 4.3:

- thiosulfate citrate bile and sucrose agar (TCBS);
- another appropriate solid selective medium (left to the choice of the laboratory) such as colistin polymyxin β-cellobiose agar (CPC), sodium dodecyl sulfate polymyxin B sucrose agar (SDS) or modified colistin polymyxin cellobiose agar (mCPC) media.

The two isolation media are incubated at 37 °C, then examined after 24 h ± 3 h.

4.5 Confirmation

The characteristic colonies of enteropathogenic *Vibrio* spp. isolated in 4.4 are subcultured, then confirmed by means of appropriate biochemical tests.

5 Culture media, reagents

For general laboratory practice, see ISO 7218.

NOTE On account of the large number of culture media and reagents, for clarity of the text, their composition and preparation are given in Annex B.

5.1 Enrichment medium: Alkaline saline peptone water (ASPW)

See B.1.

5.2 Solid selective isolation media

5.2.1 First medium: Thiosulfate, citrate, bile and sucrose (TCBS) agar

See B.2.

5.2.2 Second medium

Choose between:

- a) sodium dodecyl sulfate polymixin sucrose agar (SDS), see B.3.
- b) cellobiose polymixin colistin agar (CPC), see B.4;
- c) modified cellobiose polymixin colistin agar (mCPC), see B.5.

5.3 Saline nutrient agar (SNA)

See B.6.

5.4 Reagent for detection of oxidase

See B.7.

5.5 Saline triple sugar iron (TSI) agar

See B.8.

5.6 Saline medium for detection of ornithine decarboxylase (ODC)

See B.9.

5.7 Saline medium for detection of lysine decarboxylase (LDC)

See B.10.