TECHNICAL SPECIFICATION

ISO/TS 21872-1

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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*

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

Partie 1: Recherche de 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order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn.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 21872-1 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 that 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 1:
Detection of *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 the detection of the two main pathogenic *Vibrio* species causing intestinal illness in humans: *V. parahaemolyticus* and *V. cholerae*.

It 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.

NOTE 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.

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 parahaemolyticus* and *Vibrio cholerae*
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 parahaemolyticus* and *Vibrio cholerae*
determination of the presence or absence of *Vibrio parahaemolyticus* and *Vibrio cholerae*, 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 *Vibrio parahaemolyticus* and *Vibrio cholerae* requires four successive phases (see also Annex A).

NOTE *Vibrio parahaemolyticus* and *Vibrio cholerae* can 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 in order to detect the target organisms.

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 for deep frozen products, or at 41,5 °C for 6 h ± 1 h for fresh products.

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 41,5 °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), complementary to the TCBS medium, allowing the detection of *Vibrio parahaemolyticus* and *Vibrio cholerae*.

The TCBS is incubated at 37 °C, then examined after 24 h ± 3 h. The second selective medium is incubated according to the manufacturer's recommendations.

4.5 Confirmation

The presumptive colonies of *Vibrio parahaemolyticus* and *Vibrio cholerae* isolated in 4.4 are subcultured, then confirmed by means of appropriate biochemical tests.

5 Culture media and 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

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 manufacturers' instructions.

EXAMPLES Soya peptone triphenyl tetrazolium chloride agar (TSAT) and sodium dodecyl sulfate polymyxin sucrose (SDSPS) agar (mCPC, CPC, CC agars are not recommended for isolation of *V. parahaemolyticus*).

5.3 Saline nutrient agar (SNA)

See B.3.

5.4 Reagent for detection of oxidase

See B.4.

5.5 Saline triple sugar iron (TSI) agar

See B.5.

5.6 Saline medium for detection of ornithine decarboxylase (ODC)

See B.6.

5.7 Saline medium for detection of lysine decarboxylase (LDC)

See B.7.

5.8 Saline medium for detection of arginine dihydrolase (ADH)

See B.8.

5.9 Reagent for detection of β-galactosidase

See B.9.

5.10 Saline medium for detection of indole

See B.10.

5.11 Saline peptone waters

See B.11.

5.12 Sodium chloride solution

See B.12.

6 Apparatus and glassware

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

Usual microbiology laboratory equipment (see ISO 7218) and, in particular, the following.

6.1 **Incubator**, adjustable to 37 °C ± 1 °C.

6.2 **Incubator** or **water bath**, adjustable to 41,5 °C ± 1 °C.

6.3 **Water bath**, adjustable from 44 °C to 47 °C.

6.4 **Water bath**, adjustable to 37 °C ± 1 °C.

It is recommended to use water baths (6.2, 6.3 and 6.4) containing an antibacterial agent.

7 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

Sampling is not part of the method specified in this part of ISO/TS 21872. See the International Standard specific to the relevant product. If a specific International Standard does not exist, it is recommended that the relevant parties reach agreement on this subject.

8 Preparation of test sample

Prepare the test sample in accordance with the relevant part of ISO 6887, and/or ISO 8261, and an International Standard concerning the product to be examined. If a specific International Standard does not exist, it is recommended that the relevant parties reach agreement on this subject.

9 Procedure (see Annex A)

9.1 Test portion and initial suspension

For the preparation of the initial suspension, use the first enrichment medium (ASPW) specified in 5.1.

Take a test portion (x g or x ml), according to the sensitivity required, and homogenize it in $9x$ ml (or $9x$ g) of enrichment medium.

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

If the dilution and the incubation cannot be carried out on the same day, store the initial suspension until the next day at a temperature of 5 °C ± 3 °C.

In order to reduce the amount of examination work, where more than one 25 g test portion stemming from the same batch of food is to be examined, and where proof is available indicating that a mixture (gathering together the test portions) does not modify the results concerning this product in particular, the test portions may be mixed.

EXAMPLE If 10 test portions of 25 g are to be examined, it is possible to combine these 10 units in order to obtain a composite sample of 250 g and to add 2,25 l of enrichment medium.

Cell counts of *V. parahaemolyticus* and *V. cholerae* decline significantly on storage at refrigeration temperatures. Storage of samples and, to a lesser extent, of suspensions at such temperatures should be avoided where possible and should otherwise be kept to a minimum.

9.2 First selective enrichment

Incubate the initial suspension (9.1) at 37 °C for 6 h ± 1 h for deep-frozen products, or at 41,5 °C ± for 6 h ± 1 h for fresh, dried or salted products.

Care should be taken to apply the whole method to products with a high salt content, as the final salt concentration in the medium might alter the characteristics (see ISO 6887-4).

9.3 Second selective enrichment

9.3.1 Transfer 1 ml of the culture obtained in 9.2 taken from the surface into a tube containing 10 ml of ASPW (5.1).

9.3.2 Incubate the ASPW at 41,5 °C for 18 h ± 1 h.

9.4 Isolation and identification

9.4.1 From the cultures obtained in the ASPW (9.2 and 9.3.2), inoculate with a sampling loop the surface of a TCBS agar plate (5.2.1), so as to permit the development of well-isolated colonies.

Proceed likewise with the second selective isolation medium (5.2) using a new sampling loop.

9.4.2 Invert the agar plates. In the case of the TCBS agar plates (9.4.1), place in an incubator (6.1) set at 37 °C. For the second isolation medium, follow the manufacturer's instructions.

9.4.3 After 24 h ± 3 h of incubation, examine the plates (9.4.1 and 9.4.2) for the presence of typical colonies of presumptive pathogenic *Vibrio* spp. Mark their positions on the bottom of the dish.

There are two typical morphologies for colonies of *Vibrio* spp. on TCBS agar (5.2.1), as follows:

— typical colonies of *V. parahaemolyticus* are smooth, green (sucrose negative) and 2 mm to 3 mm in diameter;

— typical colonies of *V. cholerae* are smooth, yellow (sucrose positive) and 2 mm to 3 mm in diameter.

Incubate the second selective medium at the appropriate temperature for the appropriate time, then examine for the presence of colonies, which, according to their characteristics, may be considered as possible isolates of *V. parahaemolyticus* or *V. cholerae*.