

Australian/New Zealand Standard®

Food microbiology

Method 2.9: Examination for specific organisms—*Vibrio parahaemolyticus*

PREFACE

This Standard was prepared by the Joint Standards Australia/Standards New Zealand Committee FT/4, Food Microbiology, to supersede AS 1766.2.9—1991.

The objective of this revision is to provide an alternate procedure for performing the ONPG test. An additional test is also included for the differentiation of *V. parahaemolyticus* and *V. vulnificus* by including cellobiose fermentation in the confirmation procedure. These changes have been made to alleviate problems that have been experienced in the confirmation of *V. parahaemolyticus*.

The Committee reviewed ISO 8914:1990, Microbiology—General guidance for the detection of *Vibrio parahaemolyticus*, for adoption as the revision of AS 1766.2.9—1991. However, adoption of ISO 8914:1990 cannot be recommended because—

- (a) ISO methodology is to determine the presence or the absence of the organism, which is inadequate for regulatory purposes;
- (b) ISO methodology is a qualitative method whereas an Australian/New Zealand Standard should be a quantitative and reference method; and
- (c) lack of experience with the two plating media and the additional media specified in the ISO Standard.

This Standard is one of a series of methods for the microbiological examination of foods for quality control and investigative purposes and, where applicable, for checking that foods comply with regulatory specifications.

The term 'normative' has been used in this Standard to define the application of the appendix to which it applies. A 'normative' appendix is an integral part of a Standard.

METHOD

1 SCOPE This Standard sets out a method for estimating the most probable number (MPN) of *Vibrio parahaemolyticus* in foods.

2 REFERENCED DOCUMENTS The following documents are referred to in this Standard.

AS

1766 Food microbiology

1766.1.2 Method 1.2: General procedures and techniques—Preparation of dilutions

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AS

- 1766.1.6 Method 1.6: General procedures and techniques—Estimation of most probable number (MPN) of microorganisms
- 1766.3.1 Method 3.1: Examination of specific products—Meat and meat products other than poultry
- 1766.3.2 Method 3.2: Examination of specific products—Poultry
- 1766.3.3 Method 3.3: Examination of specific products—Dehydrated foods
- 1766.3.4 Method 3.4: Examination of specific products—Frozen foods
- 1766.3.5 Method 3.5: Examination of specific products—Molluscs, crustaceans and fish, and products thereof
- 1766.3.6 Method 3.6: Examination of specific products—Margarine
- 1766.3.7 Method 3.7: Examination of specific products—Heat-processed foods in hermetically sealed containers
- 1766.3.8 Method 3.8: Examination of specific products—Eggs and egg products
- 1766.5 Method 5: Preparation of media, diluents and reagents

3 PRINCIPLE Two sequential liquid selective enrichments and solid selective media are used for the isolation of presumptive *V. parahaemolyticus* and for their subsequent biochemical confirmation.

A flow diagram of the examination procedure is shown in Figure 1.

4 CULTURE MEDIA, REAGENTS AND REFERENCE CULTURES

4.1 General The culture media and reagents specified in this Clause shall be made up according to the formulations given in AS 1766.5 or those in Appendix A. The culture media listed in Clause 4.3 require an appropriate addition of sodium chloride to provide a concentration of 30 g/L NaCl after sterilization.

4.2 Media

4.2.1 *Alkaline peptone water*

4.2.2 *Thiosulfate citrate bile-salts sucrose (TCBS) agar*

4.2.3 *Salt tolerance media*—containing 0 g/L, 80 g/L and 110 g/L NaCl.

4.2.4 *Decarboxylase broth base (Moeller's) and decarboxylase broths containing lysine, ornithine and arginine*—with 10 g/L NaCl added (see Appendix A).

4.2.5 *Physiological saline*

4.2.6 *V. parahaemolyticus preservation medium*

4.3 Media containing 30 g/L sodium chloride (see Clause 4.1)

4.3.1 *Tryptone water*—containing 30 g/L NaCl.

4.3.2 *Buffered glucose broth*—containing 30 g/L NaCl.

4.3.3 *Nutrient agar*—containing 30 g/L NaCl.

4.3.4 *Triple sugar iron (TSI) agar*—containing 30 g/L NaCl.

4.3.5 *Bromcresol purple cellobiose broth*—containing 30 g/L NaCl.

4.3.6 *Peptone solution, 0.1 %*—containing 30 g/L NaCl.

4.4 Reagents

4.4.1 *Voges-Proskauer reagents*

4.4.2 *Oxidase reagent (Kovacs)*

4.4.3 *Buffered ONPG solution 0.0133 M*

4.5 Reference cultures *V. parahaemolyticus* NCTC 10884 or UQM 2200, *V. cholerae* and *V. vulnificus*. (See Appendix B for procedures for the long-term preservation of reference cultures.)

5 PREPARATION OF DILUTIONS OF FOOD PRODUCT

5.1 Diluent Peptone solution containing 30 g/L NaCl (4.3.6) should be used to prepare dilutions of the test sample.

5.2 Procedure The procedure shall be as follows:

- (a) Prepare the sample of food product for testing in accordance with the instructions given for that product in the appropriate AS 1766 Standard (see Clause 2).

NOTE: Additional methods of test are continually being added to the AS 1766 series of Australian Standards. If a required method is not listed in Clause 2, then the latest Catalogue of Standards Australia/Standards New Zealand Publications or a Standards Australia/Standards New Zealand Office should be consulted to ascertain whether the required method is available as an Australian/New Zealand Standard.

- (b) Prepare the required decimal dilutions of the prepared sample by the procedure described in AS 1766.1.2.

6 TEST PROCEDURES

6.1 Isolation of presumptive *V. parahaemolyticus* The procedure shall be as follows:

- (a) Using the most probable number (MPN) method (3-tube technique) described in AS 1766.1.6, inoculate tubes containing 10 mL of alkaline peptone water (4.2.1) with appropriate dilutions of the test sample (primary enrichment).
- (b) Prepare a set of controls for each series of tests by inoculating tubes of alkaline peptone water (4.2.1) with the reference cultures specified in Clause 4.5.
- (c) Incubate tests and control at $37 \pm 1^\circ\text{C}$ for 6 h to 8 h.
- (d) Transfer 1 mL from each tube to a fresh tube of alkaline peptone water (4.2.1) (secondary enrichment). Streak a loopful of each primary enrichment culture on to a prepared plate of TCBS agar (4.2.2) to obtain isolated colonies.

Each individual primary and secondary enrichment culture shall carry a unique identification code in order to determine which secondary enrichment corresponds to each primary enrichment culture when selecting colonies for confirmation and when calculating the most probable number of *V. parahaemolyticus* present.

- (e) Incubate the secondary enrichments and the TCBS agar plates at $37 \pm 1^\circ\text{C}$ for $18 \pm 2\text{h}$.
- (f) Streak a loopful of each secondary enrichment on to a prepared plate of TCBS agar (4.2.2) to obtain isolated colonies.
- (g) Incubate the TCBS agar plates at $37 \pm 1^\circ\text{C}$ for $18 \pm 2\text{h}$.
- (h) At the end of their respective incubation periods, examine the plates for typical colonies of *V. parahaemolyticus*.

6.2 Confirmation of *V. parahaemolyticus* The procedure shall be as follows:

- (a) Select three typical *V. parahaemolyticus* colonies from the TCBS agar plates streaked from each pair of primary and secondary enrichment cultures. Subculture each colony into a tube of tryptone water containing 30 g/L NaCl (4.3.1).
- (b) Incubate at $37 \pm 1^\circ\text{C}$ until faintly turbid, usually 2 h to 4 h.
- (c) Inoculate each of the following with a drop of the culture from Step (b):
 - (i) Lysine, ornithine and arginine decarboxylase broths (4.2.4) and decarboxylase broth base (as a control).
 - (ii) Buffered glucose broth containing 30 g/L NaCl (4.3.2).