

ISO/TS 21872-2:2020 (E)

Microbiology of the food chain — Horizontal method for the determination of *Vibrio* spp. — Part 2: Enumeration of total and potentially enteropathogenic *Vibrio parahaemolyticus* in seafood using nucleic acid hybridization

Contents

	Foreword
	Introduction
1	Scope
2	Normative references
3	Terms and definitions
4	Principle
4.1	General
4.2	Plating
4.3	Preparation of membranes
4.4	Hybridization
4.5	Detection
5	Culture media and reagents
5.1	General
5.2	Hybridization reagents
5.2.1	Hybridization buffer
5.2.2	Digoxigenin-labelled oligoprobes
5.2.3	Control materials
6	Equipment and consumables
7	Sampling
8	Procedure
8.1	General
8.2	Preparation of the sample
8.3	Plating
8.4	Preparation of process control
8.5	Preparation of membranes
8.5.1	Colony lift
8.5.2	Cross-linking of DNA to the membrane
8.5.3	Proteinase K treatment
8.6	Hybridization
8.6.1	General
8.6.2	Hybridization of tdh and trh probes
8.6.2.1	Pre-hybridization
8.6.2.2	Hybridization with tdh and trh probes
8.6.2.3	Stringency washes for tdh and trh detection
8.6.3	Colorimetric detection
8.6.4	Enumeration of tdh and/or trh positive colonies
8.6.5	Isolation of colonies (optional)
8.6.6	Hybridization of toxR probe
8.6.6.1	General
8.6.6.2	Pre-hybridization
8.6.6.3	Hybridization with toxR probe
8.6.6.4	Stringency washes for toxR detection
8.6.7	Colorimetric detection

8.6.8	Enumeration of toxR positive colonies
9	Interpretation of the results
10	Expression of results
11	Test report
Annex A	(normative) Flow diagram for the enumeration of total and potentially enteropathogenic <i>Vibrio parahaemolyticus</i> in seafood using nucleic acid hybridization
Annex B	(normative) Culture media and reagents
B.1	Culture media
B.1.1	Diluents
B.1.2	Saline tryptone soya agar (TSA-S)[8]
B.1.2.1	Composition
B.1.2.2	Preparation
B.1.3	Performance testing for the quality assurance of the culture media
B.2	Hybridization reagents
B.2.1	Nylon membranes for colony hybridization
B.2.2	Maas I solution (Lysis solution)
B.2.2.1	Composition
B.2.2.2	Preparation
B.2.3	Maas II solution (neutralizing solution)
B.2.3.1	Composition
B.2.3.2	Preparation
B.2.4	Standard saline citrate (SSC) solution 20×
B.2.4.1	Composition
B.2.4.2	Preparation
B.2.5	Standard saline citrate (SSC) solution 5×
B.2.5.1	Composition
B.2.5.2	Preparation
B.2.6	Standard saline citrate (SSC) solution 2×
B.2.6.1	Composition
B.2.6.2	Preparation
B.2.7	Standard saline citrate (SSC) solution 0,5×
B.2.7.1	Composition
B.2.7.2	Preparation
B.2.8	Proteinase K stock solution
B.2.8.1	Composition
B.2.8.2	Preparation
B.2.9	Proteinase K solution
B.2.9.1	Composition
B.2.9.2	Preparation
B.2.10	Washing buffer A
B.2.10.1	Composition
B.2.10.2	Preparation
B.2.11	Washing buffer B
B.2.11.1	Composition
B.2.11.2	Preparation
B.2.12	Maleic acid buffer
B.2.12.1	Composition
B.2.12.2	Preparation
B.2.13	Washing buffer C
B.2.13.1	Composition
B.2.13.2	Preparation
B.2.14	Blocking reagent
B.2.14.1	Composition
B.2.14.2	Preparation
B.2.15	Blocking solution
B.2.15.1	Composition
B.2.15.2	Preparation
B.2.16	Alkaline phosphatase (AP) conjugated anti-digoxigenin antibody
B.2.17	Antibody solution — Preparation

- B.2.18 Detection buffer
 - B.2.18.1 Composition
 - B.2.18.2 Preparation
- B.2.19 Colorimetric substrate
 - B.2.19.1 Composition
 - B.2.19.2 Preparation

Annex C (informative) Hybridization of tdh, trh and toxR genes of *V. parahaemolyticus*

- C.1 General
- C.2 Performance characteristics
 - C.2.1 General
 - C.2.2 Selectivity
 - C.2.2.1 Inclusivity test
 - C.2.2.2 Exclusivity test
 - C.2.3 Sensitivity
 - C.2.3.1 Reaction sensitivity
 - C.2.3.2 Method sensitivity
 - C.2.4 Performance parameters: accuracy, linearity, repeatability
 - C.2.5 Robustness
- C.3 Procedure
 - C.3.1 Principle
 - C.3.2 Reagents
 - C.3.2.1 General
 - C.3.2.2 Reagents for hybridization
 - C.3.2.2.1 Hybridization buffer
 - C.3.2.2.1.1 Composition
 - C.3.2.2.1.2 Preparation
 - C.3.2.2.2 Oligoprobes
- C.4 Molecular procedure
 - C.4.1 Hybridization reaction with tdh and trh probes
 - C.4.2 Hybridization reaction with toxR probe
 - C.4.3 Hybridization conditions
 - C.4.4 Stringency washes conditions
- C.5 Controls
 - C.5.1 Process controls
 - C.5.1.1 Composition
 - C.5.1.2 Preparation
 - C.5.2 Hybridization controls
 - C.5.2.1 General
 - C.5.2.2 Preparation by PCR amplification
 - C.5.3 Detection control
- C.6 Limitations of the assay

Page count: 27