DIN EN ISO 21872-1:2017-10 (E)

Microbiology of the food chain - Hor izontal method for the determination of Vibrio spp. - Part 1: Detection of potentially enteropathogenic Vibrio parahaemolyticus, Vibrio cholerae and Vibrio vulnificus (ISO 21872-1:2017)

Contents

Page

European foreword 4

Foreword 5

Introduction 6

1 Scope 7

2 Normative references 7

3 Terms and definitions 8

4 Principle 8
4.1 General 8
4.2 Primary enrichment in a liquid selective medium 8
4.3 Secondary enrichment in a liquid selective medium 9
4.4 Isolation and identification 9
4.5 Confirmation 9

5 Culture media and reagents 9
5.1 Enrichment medium: alkaline saline peptone water (ASPW) 10
5.2 Solid selective isolation media 10
5.2.1 First medium: thiosulphate, citrate, bile and sucrose agar medium (TCBS) 10
5.2.2 Second medium 10
5.3 Saline nutrient agar (SNA) 10
5.4 Reagent for detection of oxidase 10
5.5 Biochemical tests 10
5.5.1 L-lysine decarboxylase saline medium (LDC) 10
5.5.2 Arginine dihydrolase saline medium (ADH) 10
5.5.3 Reagent for detection of -galactosidase 11
5.5.4 Saline medium for detection of indole 11
5.5.5 Saline peptone waters 11
5.5.6 Sodium chloride solution 11
5.6 PCR 11
5.6.1 Tris acetate EDTA buffer (TAE) (or a buffer allowing similar performance for the purpose) 11
5.6.2 Mastermix 11
5.6.3 Primers and probes 11
5.6.4 Positive control material 11
5.6.5 Negative extraction control 12

6 Equipment and consumables 12

7 Sampling 12

8 Preparation of the test sample 12

9 Procedure (See Figure A.1) 13
9.1 Test portion and initial suspension 13

9.2 Primary selective enrichment 13
9.3 Secondary selective enrichment 13
9.4 Isolation and identification 14
9.5 Confirmation 14
9.5.1 General 14
9.5.2 Selection of colonies for confirmation and preparation of pure cultures 15
9.5.3 Tests for presumptive identification 15
9.5.4 Biochemical confirmation 16
9.5.5 PCR confirmation 18
9.5.6 DNA extraction 18
9.5.7 Conventional PCR 18
9.5.8 Real-time PCR 19

10 Expression of results 19

11 Performance characteristics of the method 19
11.1 Interlaboratory study 19
11.2 Sensitivity 20
11.3 Specificity 20
11.4 LOD50 20

12 Test report 20

Annex A (normative) Diagram of procedure 21

Annex B (normative) Composition and preparation of the culture media and reagents 23

Annex C (informative) Conventional PCR for the detection of Vibrio parahaemolyticus, thermostable direct haemolysin (tdh) and thermostable direct related haemolysin (trh) genes, Vibrio cholerae and Vibrio vulnificus 29

Annex D (informative) Real-time PCR for the detection of Vibrio parahaemolyticus, thermostable direct haemolysin gene (tdh) and Vibrio vulnificus 33

Annex E (informative) Results of an interlaboratory study 35

Bibliography 38