

# DIN EN ISO 21872-1:2023-06 (E)

Microbiology of the food chain - Horizontal method for the determination of *Vibrio* spp. - Part 1: Detection of potentially enteropathogenic *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus* (ISO 21872-1:2017 + Amd 1:2023) (includes Amendment A1: 2023)

---

Contents	Page
European foreword .....	4
<b>[A1]</b> European foreword to Amendment <b>[A1]</b> .....	5
Foreword .....	6
<b>[A1]</b> Foreword to Amendment <b>[A1]</b> .....	7
Introduction .....	8
<b>1 Scope</b> .....	<b>9</b>
<b>2 Normative references</b> .....	<b>9</b>
<b>3 Terms and definitions</b> .....	<b>10</b>
<b>4 Principle</b> .....	<b>10</b>
4.1 General .....	10
4.2 Primary enrichment in a liquid selective medium .....	10
4.3 Secondary enrichment in a liquid selective medium .....	11
4.4 Isolation and identification .....	11
4.5 Confirmation .....	11
<b>5 Culture media and reagents</b> .....	<b>11</b>
5.1 Enrichment medium: alkaline saline peptone water (ASPW) .....	12
5.2 Solid selective isolation media .....	12
5.2.1 First medium: thiosulphate, citrate, bile and sucrose agar medium (TCBS) .....	12
5.2.2 Second medium .....	12
5.3 Saline nutrient agar (SNA) .....	12
5.4 Reagent for detection of oxidase .....	12
5.5 Biochemical tests .....	12
5.5.1 L-lysine decarboxylase saline medium (LDC) .....	12
5.5.2 Arginine dihydrolase saline medium (ADH) .....	12
5.5.3 Reagent for detection of $\beta$ -galactosidase .....	12
5.5.4 Saline medium for detection of indole .....	12
5.5.5 Saline peptone waters .....	12
5.5.6 Sodium chloride solution .....	12
5.6 PCR .....	13
5.6.1 Tris acetate EDTA buffer (TAE) (or a buffer allowing similar performance for the purpose) .....	13
5.6.2 Mastermix .....	13
5.6.3 Primers and probes .....	13
5.6.4 Positive control material .....	13
5.6.5 Negative extraction control .....	13
<b>6 Equipment and consumables</b> .....	<b>13</b>
<b>7 Sampling</b> .....	<b>14</b>
<b>8 Preparation of the test sample</b> .....	<b>14</b>
<b>9 Procedure (See <a href="#">Figure A.1</a>)</b> .....	<b>14</b>
9.1 Test portion and initial suspension .....	14
9.2 Primary selective enrichment .....	15

9.3	Secondary selective enrichment.....	15
9.4	Isolation and identification.....	16
9.5	Confirmation.....	16
9.5.1	General.....	16
9.5.2	Selection of colonies for confirmation and preparation of pure cultures.....	17
9.5.3	Tests for presumptive identification.....	17
9.5.4	Biochemical confirmation.....	17
9.5.5	PCR confirmation.....	19
9.5.6	DNA extraction.....	20
9.5.7	Conventional PCR.....	20
9.5.8	Real-time PCR.....	21
<b>10</b>	<b>Expression of results.....</b>	<b>21</b>
<b>11</b>	<b>Performance characteristics of the method.....</b>	<b>21</b>
11.1	Interlaboratory study.....	21
11.2	Sensitivity.....	21
11.3	Specificity.....	22
11.4	LOD <sub>50</sub> .....	22
<b>12</b>	<b>Test report.....</b>	<b>22</b>
<b>Annex A</b>	<b>(normative) Diagram of procedure.....</b>	<b>23</b>
<b>Annex B</b>	<b>(normative) Composition and preparation of the culture media and reagents.....</b>	<b>25</b>
<b>Annex C</b>	<b>(informative) Conventional PCR for the detection of <i>Vibrio parahaemolyticus</i>, thermostable direct haemolysin (<i>tdh</i>) and thermostable direct related haemolysin (<i>trh</i>) genes, <i>Vibrio cholerae</i> and <i>Vibrio vulnificus</i>.....</b>	<b>34</b>
<b>Annex D</b>	<b>(informative) Real-time PCR for the detection of <i>Vibrio parahaemolyticus</i>, thermostable direct haemolysin gene (<i>tdh</i>) and <i>Vibrio vulnificus</i>.....</b>	<b>38</b>
<b>Annex E</b>	<b>(informative) Results of an interlaboratory study.....</b>	<b>40</b>
<b>Bibliography</b>	<b>.....</b>	<b>43</b>