

# ISO/TS 17919:2013-11 (E)

## Microbiology of the food chain - Polymerase chain reaction (PCR) for the detection of food-borne pathogens - Detection of botulinum type A, B, E and F neurotoxin-producing clostridia

---

<b>Contents</b>		<b>Page</b>
Foreword .....		iv
Introduction .....		v
<b>1</b>	<b>Scope .....</b>	<b>1</b>
<b>2</b>	<b>Normative references .....</b>	<b>1</b>
<b>3</b>	<b>Terms and definitions .....</b>	<b>1</b>
<b>4</b>	<b>Symbols and abbreviated terms .....</b>	<b>1</b>
4.1	Symbols .....	1
4.2	Abbreviated terms .....	2
<b>5</b>	<b>Principle .....</b>	<b>2</b>
5.1	General .....	2
5.2	Microbial enrichment .....	2
5.3	Nucleic acid extraction .....	2
5.4	Amplification by PCR .....	2
5.5	Detection of PCR products .....	2
5.6	Confirmation .....	2
<b>6</b>	<b>Reagents .....</b>	<b>3</b>
6.1	General .....	3
6.2	Culture media .....	3
6.3	Nucleic acid extraction .....	4
6.4	Reagents for PCR .....	5
<b>7</b>	<b>Apparatus and equipment .....</b>	<b>5</b>
7.1	General .....	5
7.2	Equipment for sample preparation prior to enrichment .....	5
7.3	Equipment for microbial enrichment .....	5
7.4	Equipment used for nucleic acid extraction .....	6
7.5	Equipment used for PCR .....	6
7.6	Equipment used for the detection of the PCR product .....	6
<b>8</b>	<b>Sampling .....</b>	<b>7</b>
<b>9</b>	<b>Procedure .....</b>	<b>7</b>
9.1	Sample preparation prior to enrichment .....	7
9.2	Microbial enrichment .....	7
9.3	Nucleic acid preparation .....	8
9.4	PCR amplification .....	9
9.5	Confirmation of a positive PCR result .....	9
<b>Annex A (normative)</b>	<b>Flow-diagram of the procedure .....</b>	<b>10</b>
<b>Annex B (informative)</b>	<b>Multiplex PCR assays for detection of genes encoding botulinum neurotoxin types A, B, E, and F using agarose gel electrophoresis .....</b>	<b>11</b>

<b>Annex C (informative) Assays for detection of genes encoding botulinum neurotoxin types A, B, E, and F using real-time PCR .....</b>	<b>29</b>
<b>Annex D (informative) Preparation of <i>C. botulinum</i> spores .....</b>	<b>40</b>
<b>Bibliography .....</b>	<b>46</b>