

ISO/TS 18867:2015-09 (E)

Microbiology of the food chain - Polymerase chain reaction (PCR) for the detection of food-borne pathogens - Detection of pathogenic *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*

Contents		Page
Foreword		v
Introduction		vi
1	Scope	1
2	Normative references	1
3	Terms and definitions	1
4	Principles	1
4.1	General	1
4.2	Microbial enrichment	2
4.3	Nucleic acid extraction	2
4.4	Amplification and detection	2
4.5	Isolation	2
5	Reagents	2
5.1	General	2
5.2	Culture media	2
5.2.1	General	2
5.2.2	Diluent	2
5.2.3	Enrichment media	2
5.2.4	Selective solid medium	4
5.2.5	Potassium hydroxide in saline solution, KOH	5
5.3	Nucleic acid extraction	5
5.4	Reagents for PCR	5
5.5	Primers and probes	5
6	Apparatus and equipment	5
6.1	General	5
6.2	Equipment for sample preparation prior to enrichment	6
6.3	Equipment for microbial enrichment	6
6.4	Equipment for nucleic acid extraction	6
6.5	Equipment for real-time PCR	6
7	Sampling	6
8	Procedure	6
8.1	Sample preparation prior to enrichment	6
8.1.1	General	6
8.1.2	Preparation of the sample	6
8.2	Microbial enrichment	7
8.2.1	Pathogenic <i>Y. enterocolitica</i>	7
8.2.2	<i>Y. pseudotuberculosis</i>	7
8.2.3	Pathogenic <i>Y. enterocolitica</i> and <i>Y. pseudotuberculosis</i>	7
8.3	Isolation of colonies, optional	7
8.3.1	Pathogenic <i>Y. enterocolitica</i>	7
8.3.2	<i>Y. pseudotuberculosis</i>	7

8.3.3	Process controls	8
8.4	Nucleic acid extraction	8
8.5	PCR amplification	8
8.5.1	General	8
8.5.2	PCR controls	8
8.6	Confirmation of the PCR product	8
8.6.1	General	8
8.6.2	Interpretation of the PCR result	8
9	Test report	9
Annex A (normative) PCR detection and isolation of pathogenic <i>Y. enterocolitica</i> (see Figure A.1)		10
Annex B (informative) Real-time PCR for detection of <i>Y. enterocolitica</i>		11
Annex C (informative) Detection and isolation of <i>Y. pseudotuberculosis</i>		21
Annex D (informative) Simultaneous detection of pathogenic <i>Y. enterocolitica</i> and <i>Y. pseudotuberculosis</i> using multiplex real-time PCR		26
Bibliography		29