DIN EN ISO 10272-2:2023-07 (E)

Microbiology of the food chain - Horizontal method for detection and enumeration of Campylobacter spp. - Part 2: Colony-count technique (ISO 10272-2:2017 + Amd 1:2023) (includes Amendment A1:2023)

Contents

Page

European foreword 5

!European foreword to Amendment A1" 6

Foreword 7

!Foreword to Amendment A1" 8

Introduction 9

1 Scope 10

2 Normative references 10

3 Terms and definitions 10

4 Principle 11
4.1 General 11
4.2 Preparation of dilutions 11
4.3 Enumeration 11
4.4 Confirmation 11

5 Culture media and reagents 11

6 Equipment and consumables 12

7 Sampling 12

8 Preparation of test sample 12

9 Procedure 13
9.1 Test portion, initial suspension and dilutions 13
9.2 Inoculation and incubation 13
9.3 Enumeration of characteristic colonies 13
9.4 Confirmation of Campylobacter 13
9.4.1 General 13
9.4.2 Selection of colonies for confirmation 14
9.4.3 Examination of morphology and motility 14
9.4.4 Study of aerobic growth at 25 °C 14
9.4.5 Detection of oxidase activity 14
9.4.6 Interpretation 14
9.5 Identification of Campylobacter species (optional) 15
9.5.1 General 15
9.5.2 Detection of catalase activity 15
9.5.3 Detection of hippurate hydrolysis 15
9.5.4 Detection of indoxyl acetate hydrolysis 16
9.5.5 Interpretation 16

10 Expression of results 16

11 Performance characteristics of the method 16
11.1 Interlaboratory study 16
11.2 Repeatability limit 16
11.3 Reproducibility limit 17

12 Test report 18

Annex A (normative) Diagram of procedure 19

Annex B (normative) Culture media and reagents 20

B.1 General 20
B.2 Diluent 20
B.3 Modified charcoal cefoperazone deoxycholate agar (mCCD agar) 20
B.3.1 Basic medium 20
B.3.2 Antibiotic solution 21
B.3.3 Complete medium 21
B.4 Columbia blood agar 21
B.4.1 Basic medium 21
B.4.2 Sterile sheep or horse blood 22
B.4.3 Complete medium 22
B.5 Reagent for the detection of oxidase activity 22
B.5.1 Composition 22
B.5.2 Preparation 22
B.6 Reagent for the detection of catalase activity 22
B.6.1 Composition 22
B.6.2 Preparation 22
B.7 Reagents for the detection of hydrolysis of hippurate 23
B.7.1 Sodium hippurate solution 23
B.7.2 Ninhydrin solution, mass fraction of 3,5 % 23
B.8 Indoxyl acetate discs 23
B.8.1 Composition 23
B.8.2 Preparation 23
B.9 Performance testing for the quality assurance of the culture media 24

Annex C (informative) Method validation studies and performance characteristics 25

!Annex D (informative) Multiplex real-time PCR assay for confirmation of thermotolerant Campylobacter spp 28

D.1 General 28
D.2 Principle 28
D.3 Reagents 28
D.3.1 Reagents for nucleic acid extraction 28
D.3.2 Reagents for real-time PCR 28
D.4 Apparatus 29
D.4.1 Equipment used for nucleic acid extraction 29
D.4.2 Equipment used for real-time PCR 29
D.5 Procedure 30
D.5.1 Nucleic acid extraction 30
D.5.2 PCR set-up 30
D.5.3 PCR controls 30
D.5.4 Temperature-time programme 30
D.6 Interpretation of the results 31
D.7 Performance characteristics of the method 31
D.7.1 General 31
D.7.2 Theoretical evaluation of the method 31
D.7.3 Inclusivity and exclusivity 31

Annex E (informative) PCR methods for molecular confirmation and identification of thermotolerant Campylobacter spp 33

E.1 General 33
E.2 Gel-based multiplex PCR assay for confirmation and identification of thermotolerant Campylobacter spp 33
E.2.1 General 33
E.2.2 Principle 33
E.2.3 Reagents 33
E.2.4 Apparatus 35
E.2.5 Procedure 36
E.2.6 Interpretation of the results 38
E.2.7 Performance characteristics 38
E.3 Multiplex real-time PCR assay for confirmation and identification of thermotolerant Campylobacter spp 39
E.3.1 General 39
E.3.2 Principle 40
E.3.3 Reagents 40
E.3.4 Apparatus 41
E.3.5 Procedure 42
E.3.6 Interpretation of the results 43
E.3.7 Performance characteristics 43

Annex F (informative) Method validation studies and performance characteristics 45

F.1 Interlaboratory study for the multiplex real-time PCR assay for confirmation of thermotolerant Campylobacter spp. (see Annex D) 45
F.2 Interlaboratory study for the gel-based multiplex PCR assay for confirmation and identification of thermotolerant Campylobacter spp. (see Clause E.2) 45
F.3 Interlaboratory study for the real-time PCR assay for confirmation and identification of thermotolerant Campylobacter spp. (see Clause E.3)" 46

Bibliography 48