

# 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)

---

<b>Contents</b>		<b>Page</b>
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 <i>Campylobacter</i> .....	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 <i>Campylobacter</i> 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