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SIST EN ISO 10272-2:2017/oprA1:2022

01-februar-2022

Mikrobiologija v prehranski verigi - Horizontalna metoda za ugotavljanje prisotnosti in števila Campylobacter spp. - 2. del: Tehnika štetja kolonij - Dopolnilo A1: Vključitev metod za molekularno potrditev in identifikacijo termotolerantnih bakterij Campylobacter spp. ter popravek preskušanja učinkovitosti gojišč (ISO 10272-2:2017/DAM 1:2021)

Microbiology of the food chain - Horizontal method for detection and enumeration of Campylobacter spp. - Part 2: Colony-count technique - Amendment 1: Inclusion of methods for molecular confirmation and identification of thermotolerant Campylobacter spp. and correction of the performance testing of the media (ISO 10272-2:2017/DAM 1:2021)

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Mikrobiologie der Lebensmittelkette - Horizontales Verfahren zum Nachweis und zur Zählung von Campylobacter spp. - Teil 2: Koloniezählverfahren - Änderung 1 (ISO 10272 2:2017/DAM 1:2021)

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Microbiologie de la chaîne alimentaire - Méthode horizontale pour la recherche et le dénombrement de Campylobacter spp. - Partie 2: Technique par comptage des colonies - Amendement 1: Ajout de méthodes de confirmation et d’identification moléculaires de Campylobacter spp. thermotolérants, et correction des essais de performance des milieux (ISO 10272-2:2017/DAM 1:2021)

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DRAFT AMENDMENT
ISO 10272-2:2017/DAM 1

ISO/TC **34**/SC **9** Secretariat: **AFNOR**

Voting begins on: **2021-11-23** Voting terminates on: **2022-02-15**

Microbiology of the food chain — Horizontal method for detection and enumeration of Campylobacter spp. —

Part 2: **Colony-count technique**

AMENDMENT 1: Inclusion of methods for molecular confirmation and identification of thermotolerant Campylobacter spp. and correction of the performance testing of the media

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Microbiologie de la chaîne alimentaire — Méthode horizontale pour la recherche et le dénombrement de Campylobacter spp. —

Partie 2: Technique par comptage des colonies

AMENDEMENT 1

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CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
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Microbiology of the food chain — Horizontal method for detection and enumeration of Campylobacter spp. —

Part 2: Colony-count technique

AMENDMENT 1: Inclusion of methods for molecular confirmation and identification of thermotolerant Campylobacter spp. and correction of the performance testing of the media

3.1

Replace the text with the following:

Campylobacter

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genus of microorganisms of the family *Campylobacteraceae*, forming characteristic colonies on solid selective media, like modified Charcoal Cefoperozone Deoxycholate agar (mCCD agar) agar, when incubated in a microaerobic atmosphere at 41,5 °C and displaying certain characteristics with biochemical confirmation tests and by microscopy.

Note 1 to entry Microscopy, the biochemical confirmation tests and the characteristics of *Campylobacter* are described in 9.4.

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Note 2 to entry This document targets the thermotolerant *Campylobacter* species relevant to human health. The most frequently encountered and relevant to human health are *Campylobacter jejuni* and *Campylobacter coli*. However, other species have been described (*Campylobacter lari, Campylobacter upsaliensis* and others).

9.4.1

Add the following text after the last paragraph:

PCR tests for confirmation and species identification are described in Annex D and E.

9.5.1, second sentence

Replace the text with the following:

However, other species have been described *(Campylobacter lari*, *Campylobacter upsaliensis* and others); the characteristics given in Table 2 permit their differentiation from *Campylobacter jejuni* and *Campylobacter coli*.

9.5.1

Add the following text as the third paragraph:

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Additionally, Annex D and E describe molecular methods for confirmation and identification of thermotolerant *Campylobacter* species, which can be used as an alternative to the biochemical identification described in 9.5.2 to 9.5.5.

9.5.4, second sentence

Replace the text with the following:

If the indoxyl acetate is hydrolysed, a colour change to blue occurs within 5 min to 10 min. If there is an unclear result after 10 min., a better result can be obtained after waiting for another 20 min. No colour change indicates hydrolysis has not taken place.

9.5.5

Replace the table with the following table:

Characteristic	*C. jejuni*	*C. coli*	*C. lari*[b]	*C. upsaliensis*[b]
Catalase (9.5.2)	+	+	+	– or weak
Hydrolysis of hippurate (9.5.3)	+[a]	–	–	–
Indoxyl acetate (9.5.4)	+	+	–	+[c]

Key: + = positive; – = negative.

[a] Some hippurate-negative *C. jejuni* strains have been reported.

[b] The same characteristics can appear also for other *Campylobacter* spp.

[c] Indoxyl acetate negative *C. upsaliensis* strains have been reported.

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11.1

Add after the first sentence the following:

The results have been published [18].

B.2

Replace the text with the following:

See ISO 6887.

B.9, Table B.1

Replace by the following Table:

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Table B.1 — Performance testing of culture media for *Campylobacter*

Medium	Function	Incubation	Control strains	WDCM numbers[a]	Reference media	Method of control	Criteria[c]	Charac-ter-istic reactions of target microor-ganism
mCCD agar	Productivity	(44 ± 4) h/ (41,5 ± 1) °C microaerobic atmosphere	*Campylobacter jejuni*[d] *Campylobacter coli*	00156 or 00005 00004	Blood agar	Quantita-tive	$P_R \geq 0,5$	Greyish, flat and moist, sometimes with me-tallic sheen
	Selectivity		*Escherichia coli*[d]	00012 or 00013	—	Qualitative	Total or partial inhibition (0–1)	No char-ac-teristic colonies
			Staphylococcus aureus[d]	00032 or 00034	—	Qualitative	Total inhibition (0)	—
Columbia blood agar	Productivity	24 h to 48 h/ (41,5 ± 1) °C microaerobic atmosphere	*Campylobacter jejuni*[d] *Campylobacter coli*[d]	00156 or 00005 or 00004	Media batch blood agar already validated	Qualitative	Good growth	—
					Media batch mCCDA already validated	Qualitative	$P_R \geq 0,7$	

[a] WDCM: World Data Centre for Microorganisms. Refer to the reference strain catalogue available at www.wfcc.info for information on culture strain numbers and contact details[10].

[b] Not applicable.

[c] Growth is categorized as 0: no growth; 1: weak growth; 2: good growth. P_R = productivity ratio (see ISO 11133).

[d] Strain free of choice, one of the strains has to be used as a minimum.

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After Annex C

Add the following as Annexes D, E and F:

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Annex D
(informative)

Multiplex real-time-PCR assay for confirmation of thermotolerant *Campylobacter* spp.

D.1 Introduction

This annex describes a probe-based multiplex real-time PCR method based on 5' exonuclease activity for the detection of a fragment of the 16S rRNA of thermotolerant *Campylobacter* spp. This assay can be used for confirmation of thermotolerant *Campylobacter* spp. [19].

D.2 Principle

A specific fragment of the 16S rRNA of thermotolerant *Campylobacter* spp. is amplified by multiplex-real-time-PCR. The PCR product is detected by measuring fluorescence of the hydrolysed probe.

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D.3 Reagents

D.3.1 General

For quality of reagents used, see ISO 22174 [21]. Ready-to-use reagents may be commercially available. The manufacturer's instructions for use should be considered. All information concerning commercially available products in the document is given for convenience of users of this document and does not constitute an endorsement by ISO of the product names. Equivalent products from other manufacturers may be used if they can be shown to give equivalent or better results. If necessary, adapt the amounts of the reagents and the temperature-time programme.

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D.3.2 Reagents for nucleic acid extraction

D.3.2.1 NaCl, 0,9 %.

D.3.2.2 PCR grade Water.

D.3.2.3 TE-buffer.

D.3.3 Reagents for real-time-PCR

D.3.3.1 PCR grade Water.

D.3.3.2 PCR buffer solution, 10 x.

The PCR buffer solution is usually delivered with the DNA polymerase, which may or may not include $MgCl_2$ in a concentration specified by the manufacturer. The final $MgCl_2$ concentration is method specific and therefore listed in Table D.2.

D.3.3.3 $MgCl_2$ solution.

D.3.3.4 Thermostable *Taq* DNA polymerase (for hot-start PCR).

D.3.3.5 dNTP solution.

D.3.3.6 Oligonucleotides.

Sequences of the oligonucleotides are listed in Table D.1.

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D.3.3.7 ntb2-plasmid.

A vector plasmid carrying a 125-bp sequence of the gene *rbcMT-T* encoding Ribulose-1,5-bisphosphate carboxylase/oxygenase N-methyltransferase from *Nicotiana tabacum* [26].

Table A.1 — Sequences of oligonucleotides

gene	Primer/probe	Sequence (5´ — 3´)
16S rRNA	Jos-F1	CCT GCT TAA CAC AAG TTG AGT AGG
	Jos-R1	TTC CTT AGG TAC CGT CAG AAT TC
	Jos-P	FAM[a]- TGT CAT CCT CCA CGC GGC GTT GCT GC-NFQ[b]
Internal Amplification Control (IAC)	IPC-ntb2-fw	ACC ACA ATG CCA GAG TGA CAA C
	IPC-ntb2-re	TAC CTG GTC TCC AGC TTT CAG TT
	IPC-ntb2-probe	ROX[a]-CAC GCG CAT GAA GTT AGG GGA CCA-NFQ[b]

[a] Equivalent reporter dyes and/or quencher dyes may be used for the probes if they can be shown to yield similar or better results. The alternative combinations FAM-HEX, FAM-TAMRA, FAM-JOE and FAM-Cy5 have been used with equivalent result in the validation of the method.

[b] NFQ: Non-fluorescence quencher (dark quencher)

D.4 Apparatus

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D.4.1 General

Appropriate equipment according to the method and, in particular, the following.

D.4.2 Equipment used for nucleic acid extraction

D.4.2.1 Microcentrifuge tubes, of capacities of 1,5 ml and 2,0 ml.

D.4.2.2 Thermo block, with a mixing frequency between 300 rpm and 1 400 rpm.

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D.4.2.3 Pipettes and pipette filter tips, for volumes between 1 µl and 1 000 µl.

D.4.2.4 Centrifuge, for reaction tubes having a capacity of 1,5 ml and 2,0 ml, e.g. microcentrifuge, capable of achieving an acceleration of up to 12 000 × g. In some steps a refrigerated centrifuge is required.

D.4.3 Equipment used for real-time-PCR

D.4.3.1 Pipettes and pipette filter tips, having a capacity between 1 µl and 1 000 µl.

D.4.3.2 Microcentrifuge tubes, having a capacity of 1,5 ml and 2,0 ml.

D.4.3.3 Thin-walled PCR microtubes, 0,2 ml or 0,5 ml reaction tubes, multi-well PCR microplates or other suitable consumables.

D.4.3.4 Real-time PCR instrument.

D.5 Procedure

D.5.1 Nucleic acid extraction

One 1 µl-loop of suspected colonies is (see 9.5.2.2) suspended in 1 ml of 0,9 % NaCl solution and DNA is extracted with a thermal lysis step (15 min at 95° C). After an additional centrifugation step for 3 min at 10 000 × g 5 µl of the supernatant is used as DNA template. If the DNA will be stored, TE-buffer should be used instead of 0,9 % NaCl. Other methods for DNA extraction can be used if they have been shown to be suitable. Before addition to the PCR mastermix, the template should be 100-fold diluted in sterile water.

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