SLOVENSKI STANDARD
SIST EN ISO 10272-1:2017/oprA1:2022

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

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

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

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

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DRAFT AMENDMENT
ISO 10272-1:2017/DAM 1

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

Part 1: Detection method

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

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

Partie 1: Méthode de recherche

AMENDEMENT 1

This document is circulated as received from the committee secretariat.

ISO/CEN PARALLEL PROCESSING

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This document was prepared by the European Committee for Standardization (CEN) Technical Committee CEN/TC 463, Microbiology of the food chain, in collaboration with ISO Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

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

Part 1: Detection method

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

genus of microorganisms of the family *Campylobacteraceae*, usually capable of growth, in the selective enrichment media Bolton broth and Preston broth, forming characteristic colonies on solid selective media, like modified Charcoal Cefoperozone Deoxycholate agar (mCCD 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 9.5.

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).

4.1

Add the following note after the last paragraph:

Note 1 to entry The enrichment broth used in Detection procedure B (Preston broth) may be too selective to allow the recovery of some strains of *C. coli*, see ISO 17995 [18]. The addition of growth supplement to Preston broth enhances the recovery of *Campylobacter* spp. and some strains will not grow without it. This issue arose after the publication of ISO 10272-1:2017 and is due to properties of the antibiotic solution. Supporting data are available at: https://standards.iso.org/iso/10272/-1/ed-1/en/amd/1/.

9.5.1

Add the following text after the last paragraph:

PCR tests for confirmation and species identification are described in Annex D and E. The results for the ILS study are described in Annex F.

9.6.1, 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*.

9.6.1

Add the following text as third sentence:

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 9.6.2 to 9.6.5.

9.6.4, second paragraph

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.

9.6.5, Table 2

Replace the table with the following:

Characteristic	*C. jejuni*	*C. coli*	*C. lari*[b]	*C. upsaliensis*[b]
Catalase (9.6.2)	+	+	+	– or weak
Hydrolysis of hippurate (9.6.3)	+[a]	–	–	–
Indoxyl acetate (9.6.4)	+	+	–	+[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.

11.1

Add after the first sentence the following:

The results have been published [19].

B.3

Replace the clause with the following:

B.3 Preston broth

B.3.1 Basic medium

B.3.1.1 Composition

Enzymatic digest of animal tissues		10,0 g
Peptone		10,0 g
Sodium chloride	(CAS No. 7647-14-5)	5,0 g
Water		940 ml

B.3.1.2 Preparation

Dissolve the basic components or the dehydrated complete basic medium in the water, by heating if necessary.

Adjust the pH, if necessary, so that after sterilization, the pH of the complete medium is 7,4 ± 0,2 at 25 °C. Dispense the basic medium into containers of suitable capacity. Sterilize in the autoclave set at 121 °C for 15 min.

B.3.2 Sterile lysed horse blood

Use horse blood saponin-lysed or lysed by freezing then thawing.

B.3.3 Antibiotic solution

B.3.3.1 Composition

Polymyxin B sulfate	(CAS No. 1405-20-5)	5 000 IU
Rifampicin	(CAS No. 13292-46-1)	0,01 g
Trimethoprim lactate salt	(CAS No. 23256-42-0)	0,01 g
Amphotericin B	(CAS No. 1397-89-3)	0,01 g
Ethanol, 95 % (volume fraction)		5 ml

B.3.3.2 Preparation

Dissolve the components in the ethanol.

B.3.4 Growth supplement

B.3.4.1 Composition

Sodium pyruvate	(CAS No. 113-24-6)	0,25 g
Sodium metabisulfite	(CAS No. 7681-57-4)	0,25 g
Iron(II) sulfate hydrate	(CAS No. 13463-43-9)	0,25 g
Water		5 ml

B.3.4.2 Preparation

Dissolve the components in the water and sterilize by filtration. Aliquots of 5 ml should be stored at (–20 ± 5) °C for not more than twelve months.

B.3.5 Complete medium

B.3.5.1 Composition

Basic medium (B.3.2)	940 ml
Sterile lysed horse blood (B.3.2)	50 ml
Antibiotic solution (B.3.3)	5 ml
Growth supplement (B.3.4)	5 ml

B.3.5.2 Preparation

To the basic medium, cooled down to below 47 °C, add the antibiotic solution and then the growth supplement and finally the lysed blood aseptically, and mix. Dispense the medium aseptically into tubes, bottles or flasks of suitable capacity to obtain the portions necessary for the test. If the enrichment medium has been prepared in advance, store it in the dark at 5 °C (6.7) for up to seven days.

B.11, Table B.1

Replace Table B.1 with the following table:

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

Medium	Function	Incubation	Control strains	WDCM numbers[a]	Method of control	Criteria[c]	Characteristic reactions of target microorganism
Bolton broth	Productivity	(5 ± 1) h / (37 ± 1) °C then (44 ± 4) h / (41,5 ± 1) °C microaerobic atmosphere	*Campylobacter jejuni*[d]	00156 or 00005	Qualitative	>10 characteristic colonies on mCCD agar	Greyish, flat and moist, sometimes with metallic sheen
			+ *Escherichia coli*[d]	00012 or 00013			
			+ *Staphylococcus aureus*[d]	00032 or 00034			
			Campylobacter coli	00004			
			+ *Escherichia coli*[d]	00012 or 00013			
			+ *Staphylococcus aureus*[d]	00032 or 00034			
	Selectivity		*Escherichia coli*[d]	00012 or 00013	Qualitative	< 10 colonies on TSA	—
			Staphylococcus aureus[d]	00032 or 00034			

[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[17].

[b] Not applicable.

[c] Growth/turbidity is categorized as 0: no growth/turbidity; 1: weak growth/turbidity; 2: good growth/turbidity (see ISO 11133).

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

Table B.1 *(continued)*

Medium	Function	Incubation	Control strains	WDCM numbers[a]	Method of control	Criteria[c]	Characteristic reactions of target microorganism
Preston broth	Productivity	(24 ± 2) h / (41,5 ± 1) °C microaerobic atmosphere	*Campylobacter jejuni*[d] + *Escherichia coli*[d] + *Staphylococcus aureus*[d]	00156 or 00005 00012 or 00013 00032 or 00034	Qualitative	>10 characteristic colonies on mCCD agar	Greyish, flat and moist, sometimes with metallic sheen
			Campylobacter coli + *Escherichia coli*[d] + *Staphylococcus aureus*[d]	00004 00012 or 00013 00032 or 00034			
	Selectivity		*Escherichia coli*[d]	00012 or 00013	Qualitative	Total inhibition (0) on TSA	—
			Staphylococcus aureus[d]	00032 or 00034			
mCCD agar	Productivity	(44 ± 4) h / (41,5 ± 1) °C microaerobic atmosphere	*Campylobacter jejuni*[d] *Campylobacter coli*	00156 or 00005 00004	Qualitative	Good growth (2)	Greyish, flat and moist colonies, sometimes with metallic sheen
	Selectivity		*Escherichia coli*[d]	00012 or 00013	Qualitative	Total or partial inhibition (0–1)	No characteristic 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	Qualitative	Good growth (2)	

[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[17].

[b] Not applicable.

[c] Growth/turbidity is categorized as 0: no growth/turbidity; 1: weak growth/turbidity; 2: good growth/turbidity (see ISO 11133).

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

Annex C

Add after the fourth sentence the following:

NOTE The introduction of growth supplement to the composition of Preston broth (see 4.1) does not have an impact on the results of the validation study. The results are still valid and does not need to be verified [19].

After Annex C

Add the following as Annexes D, E and F: