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AMENDMENT 1
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Microbiology of the food chain — Horizontal method for the determination of *Vibrio* spp. —

Part 1: Detection of potentially enteropathogenic *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus*

AMENDMENT 1: Inclusion of performance testing of culture media and reagents

Microbiologie de la chaîne alimentaire — Méthode horizontale pour la détermination des Vibrio spp. —

Partie 1: Recherche des espèces de Vibrio parahaemolyticus, Vibrio cholerae et Vibrio vulnificus potentiellement entéropathogènes

AMENDEMENT 1: Inclusion des essais de performance des milieux de culture et réactifs

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Foreword

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This document was prepared by Technical Committee TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 463, *Microbiology of the food chain*, 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 the determination of *Vibrio* spp. —

Part 1: Detection of potentially enteropathogenic *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus*

AMENDMENT 1: Inclusion of performance testing of culture media and reagents

Clause 5

Replace the third paragraph with the following:

For performance testing of culture media and reagents, follow the procedures in accordance with ISO 11133 and Clause B.12.

5.2.1

Delete the second sentence and Table 1.

Annex B

Add the following text to the end of the annex.

B.12 Performance testing

The definition of selectivity and productivity is specified in ISO 11133. In general, follow the procedures for performance testing described in ISO 11133. Performance testing details are given in Table B.1 and Table B.2.

Table B.1 — Performance testing for the quality assurance of the culture media

Medium	Function	Incubation	Control strains	WDCM number[a]	Method of control	Criteria[c]
Alkaline saline peptone water (ASPW)	Productivity	41,5 °C ± 1 °C for 6 h ± 1 h	*Vibrio parahaemolyticus*[d] *Vibrio cholerae*[d] non-O1/non-O139[e]	00185 00203[f]	Qualitative	> 10 colonies on TCBS
		37 °C ± 1 °C for 6 h ± 1 h	*Vibrio vulnificus*[d]	00139 00187		
Thiosulfate-citrate-bile salts (TCBS) sucrose agar	Productivity	37 °C ± 1 °C for 24 h ± 3 h	*Vibrio parahaemolyticus* *Vibrio furnissii* *Vibrio cholerae* non-O1/non-O139[e]	00185[b] 00186[b] 00203[f]	Qualitative	Good growth of green colonies (2) Good growth of yellow colonies (2) Good growth of yellow colonies (2)
	Selectivity	37 °C ± 1 °C for 24 h ± 3 h	*Escherichia coli*[d]	00012 00013 00090		Total inhibition (0)
Saline nutrient agar (SNA)	Productivity	37 °C ± 1 °C for 24 h ± 3 h	*Vibrio parahaemolyticus* *Vibrio cholerae* non-O1/non-O139[e] *Vibrio vulnificus*[d]	00185[b] 00203[f] 00139 00187	Qualitative	Good growth (2)

[a] World Data Centre for Microorganisms (WDCM) strain catalogue, available from: https://refs.wdcm.org/.

[b] Strain to be used as a minimum (see ISO 11133).

[c] Growth is categorized as 0: no growth, 1: weak growth (partial inhibition) and 2: good growth (see ISO 11133).

[d] Strain free of choice; one of the strains has to be used as a minimum (see ISO 11133).

[e] The use, storage and transport of *V. cholerae* can be limited by some national restrictions and directions.

[f] An appropriate sucrose-positive *Vibrio* species other than *V. cholerae* WDCM 00203 can also be used.

Table B.2 — Performance testing of confirmation media and reagents

Medium/reagent	Function	Control strains[a]	WDCM[b] numbers	Characteristic reactions
Arginine dihydrolase saline medium	Detection of L-Arginine dihydrolase	*Vibrio fluvialis*	00137	Positive reaction: Turbidity and violet/purple colour
		Vibrio parahaemolyticus	00037 00185	Negative reaction: Yellow colour
Tryptone/ tryptophan saline medium with indole reagent (Kovacs reagent)	Detection of indole formation from tryptophan	*Escherichia coli*	00012 00013 00090 00179	Positive reaction: Formation of a red ring within 10 min
		Vibrio parahaemolyticus	00037 00138 00185	
		Vibrio cholerae non-O1/non-O139[d]	00203[e]	
		Vibrio vulnificus	00139	
		Enterobacter aerogenes	00175	Negative reaction: Yellow/brown ring within 10 min
		Citrobacter freundii	00006	
		Salmonella enterica serovar Typhimurium[c]	00031	
Lysine decarboxylase saline medium	Detection of L-Lysine decarboxylase (LDC)	*Salmonella enterica* serovar Typhimurium[c]	00031	Positive reaction: Medium remains purple after incubation and is turbid
		Salmonella enterica serovar Enteritidis[c]	00030	
		Enterobacter aerogenes	00175	
		Vibrio parahaemolyticus	00185	
		Proteus mirabilis	00023	Negative reaction: Medium changes from purple to yellow
		Citrobacter freundii	00006	
		Cronobacter sakazakii	00214	
		Cronobacter muytjensis	00213	
		Escherichia coli	00012 00013 00090 00179	

[a] Strain free of choice; one of the strains has to be used as a minimum. The user may choose any of the strains cited for positive and negative reactions (see ISO 11133).

[b] Refer to the reference strain catalogue available on http://www.wfcc.info for information on culture collection strain numbers and contact details.[20]

[c] Some national restrictions and directions require the use of a different serovar. Refer to national requirements relating to the choice of *Salmonella* serovars.

[d] The use, storage and transport of *V. cholerae* can be limited by some national restrictions and directions.

[e] An appropriate sucrose-positive *Vibrio* species other than *V. cholerae* WDCM 00203 can also be used.

Table B.2 *(continued)*

Medium/reagent	Function	Control strains[a]	WDCM[b] numbers	Characteristic reactions
Oxidase reagent	Detection of cytochrome oxidase	*Pseudomonas aeruginosa*	00024 00025 00026	Positive reaction: Mauve, violet, purple or dark blue colour in the reaction time
		Pseudomonas fluorescens	00115	
		Vibrio parahaemolyticus	00185	
		Escherichia coli	00012 00013 00090 00179	Negative reaction: No colour change in the reaction time
		Cronobacter sakazakii	00214	
		Cronobacter muytjensis	00213	
		Brochothrix thermosphacta	00071	
Peptone waters with different NaCl concentration: 0 %, 6 %, 10 %	Detection of halotolerance 0 % NaCl	*Vibrio cholerae* non-O1/non-O139[d]	00203[e]	Positive reaction: Growth (turbidity)
		Vibrio parahaemolyticus	00185	Negative reaction: No growth (no turbidity)
	Detection of halotolerance 6 % NaCl	*Vibrio parahaemolyticus*	00037 00138 00185	Positive reaction: Growth (turbidity)
		Vibrio vulnificus	00139	
		Vibrio cholerae non-O1/non-O139[d]	00203[e]	Negative reaction: No growth (no turbidity)
	Detection of halotolerance 10 % NaCl	*Staphylococcus aureus*	00032 00034	Positive reaction: Growth (turbidity)
		Vibrio cholerae non-O1/non-O139[d]	00203[e]	Negative reaction: No growth (no turbidity)
		Vibrio parahaemolyticus	00037 00138 00185	
		Vibrio vulnificus	00139	
Saline solution with toluene and β- Galactosidase reagent	Detection of β-Galactosidase	*Escherichia coli*	00012 00013 00090 00179	Positive reaction: Yellow colour
		Proteus mirabilis	00023	Negative reaction: No colour change
		Vibrio parahaemolyticus	00185	

[a] Strain free of choice; one of the strains has to be used as a minimum. The user may choose any of the strains cited for positive and negative reactions (see ISO 11133).

[b] Refer to the reference strain catalogue available on http://www.wfcc.info for information on culture collection strain numbers and contact details.[20]

[c] Some national restrictions and directions require the use of a different serovar. Refer to national requirements relating to the choice of *Salmonella* serovars.

[d] The use, storage and transport of *V. cholerae* can be limited by some national restrictions and directions.

[e] An appropriate sucrose-positive *Vibrio* species other than *V. cholerae* WDCM 00203 can also be used.