AMENDMENT NO. 2 JANUARY 2024

TO

IS 17383 : 2020/ISO 11133 : 2014

MICROBIOLOGY OF FOOD, ANIMAL FEED AND WATER — PREPARATION, PRODUCTION, STORAGE AND PERFORMANCE TESTING OF CULTURE MEDIA

This Amendment No. 2 is identical with Amendment No. 2 of ISO 11133 : 2014 'Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media' issued in May 2020 by International Organization for Standardization (ISO).

Indian Standard

MICROBIOLOGY OF FOOD, ANIMAL FEED AND WATER — PREPARATION, PRODUCTION, STORAGE AND PERFORMANCE TESTING OF CULTURE MEDIA

AMENDMENT 2

End of 5.2 (before the NOTE)

Add the following sentence:

Annex K gives the test microorganisms to be used for confirmation media and reagents in specified food and water microbiology International Standards.

5.4.1, first sentence

Replace the sentence with the following text:

Suitable microorganisms for routine performance testing are listed in Annexes E, F and K.

End of 6.6.1

Add the following sentence:

Suitable test organisms are described in Annex K.

6.6.2, after the second sentence

Add the following sentence:

Suitable test organisms are described in Annex K.

Annex K

Add the following text as a new annex.

Annex K

(normative)

Performance testing of confirmation media and reagents

This annex specifies control strains for the performance testing of confirmation and characterization media, reagents, dyes, stains and materials described in standards for the microbiological examination of samples from the food chain and water.

For the microbiological media and reagents under test, the inoculum used is a subculture of an isolated colony. Therefore, the method of performance testing for these products is qualitative.

The shortest permissible incubation time specified in the relevant International Standard for the confirmation or characterization test should be used for the positive control organism(s), while the longest permissible incubation time should be used for the negative control organism(s).

The strains chosen in <u>Table K.1</u> have been selected preferentially from those already cited in this document. If a suitable strain was not available from this source, a strain from the catalogue of organisms compiled by the World Data Centre for Microorganisms $(WDCM)^{[20]}$ has been selected.

In most cases, more than one control strain has been listed in <u>Table K.1</u> for both positive and negative reactions. The user may choose any of the strains cited for positive and negative reactions.

If control strains for performance testing of confirmation or characterization media, reagents, dyes, stains and materials are already specified in the International Standard, for example, as in ISO 10272-1 and ISO 10272-2 (*Campylobacter*) and ISO 10273 (*Yersinia enterocolitica*), they have not been included in Table K.1. In addition, serological reagents have not been included.

If commercially sourced media or reagents are used, follow the manufacturer's instructions, including time, temperature and conditions of performance. If the instructions do not include control strains, choose a positive and a negative strain from <u>Table K.1</u>. See Clause 6 for requirements.

Table K.1 — Control strains for confirmation and characterization media, reagents, dyes, stains and materials included in documents from ISO/TC 34/SC 9, ISO/TC 34/SC 5 and ISO/TC 147/SC 4

Medium/reagent	International Standard	Function	Control strains ^a	WDCM ^b numbers	Characteristic reactions
				00024	Positive reaction:
			Pseudomonas aeruginosa	00025	Yellow to brick red after adding
Acetamide broth		Detection of ammonia		00026	1 to 2 drops of Nessler's reagent
with Nessler's	ISO 16266	production from		00012	
reagent		acetamide	Escherichia coli	00013	Negative reaction:
			Escherichia con	00090	No yellow to brick red colour
				00179	
				00012	
			Escherichia coli	00013	Positive reaction:
Acetate agar	ISO 21567	Growth on acetate	Escreticina con	00090 00179	Blue colonies with surrounding medium blue/green
(Sodium acetate agar)	150 21007	agar	Shigella sonnei	00127	Negative reaction:
			Shigella flexneri	00125	No growth or very weak growth, no colour change of the medium (remains green)
	ISO 14189	Detection of acid phosphatase	Clostridium perfringens	00007	
				00080	Positive reaction:
Acid phosphatase reagent				00174	Mauve/purple/violet colour
reagent			Clostridium bifermentans	22252	Negative reaction:
				00079	No mauve/purple/violet colour
		Detection of L-Arginine	Vibrio fluvialis		Positive reaction:
Arginine dihydrolase saline	ISO 21872-1			00137	Turbidity and violet/purple colour
medium		dihydrolase	Vibrio parahaemolyticus	00037	Negative reaction:
			Vibrio paranaemolyticus	00185	Yellow colour
				00009	
			Enterococcus faecalis	00087	Positive reaction:
				00176	Tan to black colour in the
				00177	surrounding medium
Bile aesculin azide agar			Enterococcus faecium	00177	
	ISO 7899-2	Detection of aesculin hydrolysis	Aerococcus viridans	00178	
				00012	Negative reaction:
			Escherichia coli	00013	No tan to black colour in the surrounding medium
				00090	Jan Tourium meurum
				00179	

^a Strain free of choice; one of the strains has to be used as a minimum.

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 can require the use of a different serovar. Refer to national requirements relating to the choice of Salmonella serovars.

d Weak coagulase-producing strain of S. aureus.

Table K.1 (continued)

Medium/reagent	International Standard	Function	Control strains ^a	WDCM ^b numbers	Characteristic reactions
				00012	
			Escherichia coli	00013	Positive reaction:
D. III.	ISO 4831	D	Lisener tenta con	00090	Gas produced in Durham tube
Brilliant green lactose bile broth	ISO 4832	Detection of gas production		00179	
	100 1002			00009	Negative reaction:
			Enterococcus faecalis	00087	No gas produced in Durham tube
				00176	<u> </u>
					Positive reaction:
			Listeria monocytogenes	00020	Narrow enhanced zone of β-haemolysis at the intersection
CAMP medium with		Detection of CAMP reaction		00021	of the test strain with Staphylococcus aureus.
Staphylococcus aureus WDCM 00034 and Rhodococcus equi	ISO 11290-1 ISO 11290-2		Listeria ivanovii	00018	Wide arrowhead zone of β-haemolysis at the intersection with <i>Rhodococcus equi</i>
WDCM 00028					Negative reaction:
			Listeria ivanovii	00018	Zone not enhanced with Staphylococcus aureus
			Listeria innocua	00017	No zone
				00012	
				00013	Positive reaction:
	100 11200 1		Escherichia coli	00090	Change of colour to yellow
Carbohydrate utilization broths with different carbohydrates and different indicators	ISO 11290-1 ISO 11290-2			00179	
	ISO 21567	Detection of carbohydrate		00021	
	ISO 10273	fermentation	Listeria monocytogenes	00109	Rhamnose: yellow
	ISO 22964		Proteus mirabilis		Negative reaction:
	150 22964			00023	No change in colour
			Listeria monocytogenes	00021 00109	Xylose; no change

^a Strain free of choice; one of the strains has to be used as a minimum.

 $^{^{}b} \qquad \text{Refer to the reference strain catalogue available on } \underline{\text{http://www.wfcc.info}} \text{ for information on culture collection strain numbers and contact details}^{[20]}.$

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

d Weak coagulase-producing strain of S. aureus.

 Table K.1 (continued)

Medium/reagent	International Standard	Function	Control strains ^a	WDCM ^b numbers	Characteristic reactions
			Staphylococcus aureus	00032 00034	
			Campylobacter jejuni	00005	
	100 0000		Listeria monocytogenes	00020 00021	Positive reaction: Formation of bubbles of oxygen
Catalase reagent (3 % hydrogen	ISO 9232 ISO 10272-1 ISO 10272-2	Detection of catalase after adding hydrogen	Listeria innocua	00017	
peroxide solution)	ISO 10272 2	peroxide solution	Listeria ivanovii	00018	
	ISO 11290-2			00009	
	130 112 70-2		Enterococcus faecalis	00087	
			,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	00176	Negative reaction:
			Entaro acasas faccium	00177	No formation of bubbles of oxygen
			Enterococcus faecium	00178	
			Lactobacillus delbrueckii subsp. bulgaricus	00121	
	ISO 21567	Growth on citrate agar	Enterobacter aerogenes	00175	Positive reaction:
Citrate agar			Enterobacter cloacae	00083	Cream/pink growth with surrounding medium red
(Christensen's citrate agar)			Shigella sonnei	00127	N
citrate agary			Shigella flexneri	00125	Negative reaction:
				00126	No growth
				00012	
				00013	Positive reaction:
	ICO /TC 110F0		Escherichia coli	00090	Yellow colour
Glucose agar	ISO/TS 11059			00179	
	ISO 21528-1	Production of acid from glucose		00024	
O-F medium with overlay	ISO 21528-2		Pseudomonas aeruginosa	00025	Negative reaction:
	130 21320-2		1 seudomonus dei aginosa	00026	Growth, but no yellow colour development
			Pseudomonas fluorescens	00115	
Glucose MRS broth					Positive reaction:
	100 0222	Detection of CO ₂	Lactobacillus brevis	00099	Agar layer detaches itself from the underlying contents
with overlay agar	ISO 9232	production	Lactobacillus delbrueckii		Negative reaction:
			subsp. bulgaricus	00102	No gas production, agar layer not detached

^a Strain free of choice; one of the strains has to be used as a minimum.

Refer to the reference strain catalogue available on $\frac{\text{http://www.wfcc.info}}{\text{for information on culture collection strain numbers and contact details}} [20].$

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

 $^{^{}m d}$ Weak coagulase-producing strain of S. aureus.

Table K.1 (continued)

Indole reagent (Kovacs reagent)	Medium/reagent	International Standard	Function	Control strains ^a	WDCM ^b numbers	Characteristic reactions
medium/reagent ISO 6579-1 ISO 9308-1 medium with indole reagent (Krowacs reagen) ISO 1866-1 Indole reagent (Krowacs reagen) ISO 1866-1 Indole reagent (Krowacs reagen) ISO 1866-1 Indole reagent (Krowacs reagen) ISO 1866-2 Iso 9308-1 Iso 9308-					00012	
Tryptone/ tryptophan medium with indole reagent (Kovacs reagent) Indole reagent (Kovacs reagen	Indole reaction			Escharichia cali	00013	
Tryptone/ tryptophan medium with indole reagent (Kovacs reagent) Tryptone/ tryptophan saline medium with indole reagent (Kovacs reagent) Tryptone/ tryptophan saline medium with indole reagent (Kovacs reagent) Indole reagen	medium/reagent			Escherichia con	00090	
Tryptophan medium with indole reagent (Kovacs reagent) 150 16654 (Kovacs reagent) 150 11866-1 150 11866-1 150 11866-2					00179	
tryptopham medium with indole reagent (Kowacs reagent) IND 16654 (Kowacs reagent) IND 1866-1 IND 1866-2 IN	Tryptone/	ISO 6579-1			00037	Positive reaction:
indole reagent (Kovacs reagent) Tryptone/ Tryptophan saline medium with indole reagent (Kovacs reagent) Iso 21872-1 Indole reagent (Kovacs reagent) Iso 11866-1 Iso 11866-2 Iso 11866-3 Iso 11866-3 Iso 11866-1 Iso 11866	tryptophan	ISO 9308-1		Vibrio narabaomoluticus		
Iso 21567 Detection of indole formation from tryptophan saline medium with indole reagent (Kovacs reagent)	indole reagent	ISO 16654		vibi io paranaemolyticus		
Tryptophe/ tryptophan saline medium with indole reagent (Kovacs reagent) ISO 21872-1 Indole reagent (Kovacs reagent) ISO 11866-1 Iso 11866-2 Iso 11866-1 Is	(Kovacs reagent)	ISO 21567	Detection of indole		00103	
Indole reagent (Kovacs reagent) ISO 11866-1 ISO 11866-2 ISO 1186	Tryptone/		formation from	Vibrio cholerae	00203	
Indole reagent (Kovacs reagent) ISO 11866-1 ISO 11866-1 Enterobacter aerogenes Citrobacter freundii Conomic formation on membrane filters Escherichia coli Citrobacter aerogenes Citrobacter freundii Citrobacter	medium with	ISO 21872-1	tryptophan	Vibrio vulnificus	00139	
Indole reagent (Kovacs reagent) ISO 11866-1 Iso 11866-2 Iso 11866-3 Iso 11866-3				, ,		
Citrobacter freundii Salmonella Typhimurium O0006 Negative reaction: Yellow/brown ring within 10 min Salmonella Enteritidisc O0030 O0012 O0013 O0014 O0015 O0015 O0015 O0015 O0016 O0016 O0017				Enterobacter aerogenes	00175	
Salmonella Typhimurium Salmonella Enteritidis O0030 Yellow/brown ring within 10 min Yellow/brown r		ISO 11866-1		Citrobacter freundii	00006	Negative
Indole reagent (Vracko and Sherris reagent) ISO 11866-2 Detection of indole formation on membrane filters Escherichia coli Escherichia coli Detection of indole formation on membrane filters Enterobacter aerogenes O0075 O00013 O0090 Within a few minutes O0079 O						
Indole reagent (Vracko and Sherris reagent) King's B medium ISO 16266 ISO 1866-2 Detection of indole formation on membrane filters Detection of indole formation on membrane filters Detection of indole formation on membrane filters Enterobacter aerogenes Citrobacter freundii Salmonella Typhimuriumc Salmonella Enteritidisc O0030 Pseudomonas aeruginosa O0024 Positive reaction: No pink colour development Pseudomonas aeruginosa O0025 Presence of fluorescence under UV radiation (360 ± 20) nm Escherichia coli Negative reaction: Presence of fluorescence under UV radiation (360 ± 20) nm Negative reaction: Presence of fluorescence under UV radiation (360 ± 20) nm Negative reaction: Presence of fluorescence under UV radiation (360 ± 20) nm				Salmonella Typhimurium ^c	00031	Yellow/brown ring within 10 min
Indole reagent (Vracko and Sherris reagent) Enterobacter aerogenes Citrobacter freundii Salmonella Typhimuriumc Salmonella Enteritidisc $O0031$ Negative reaction: Negative reaction: No pink colour development No pink colour development Pseudomonas aeruginosa Positive reaction: No pink colour development No pink colour development Pseudomonas aeruginosa O0025 Presence of fluorescence under UV radiation (360 \pm 20) nm Negative reaction: Presence of fluorescence under UV radiation (360 \pm 20) nm Negative reaction: No pink colour development No pink colour development O0025 Presence of fluorescence under UV radiation (360 \pm 20) nm Negative reaction:				Salmonella Enteritidis ^c	00030	
Indole reagent (Vracko and Sherris reagent) ISO 11866-2 Detection of indole formation on membrane filters Enterobacter aerogenes Citrobacter freundii Citrobacter freundii O0006 Negative reaction: No pink colour development Salmonella Typhimuriumc O0031 O0024 Positive reaction: Pseudomonas aeruginosa O0025 O0026 O0026 O0026 O0026 O0026 O0026 O0027 O00					00012	
Indole reagent (Vracko and Sherris reagent) ISO 11866-2 ISO 11866-2 ISO 11866-2 ISO 11866-2 ISO 11866-2 Detection of indole formation on membrane filters Enterobacter aerogenes Citrobacter freundii Salmonella Typhimuriumc O0031 No pink colour development Salmonella Enteritidisc O0024 Positive reaction: Presence of fluorescence under UV radiation (360 ± 20) nm Escherichia coli Peseudomonas aeruginosa O0012 O0013 Negative reaction: Presence of fluorescence under UV radiation (360 ± 20) nm No pink colour development O0025 O0026 O0017 No pink colour development O0026 O0027 O0028 Negative reaction: O0029 Negative reaction: O0010 Negative reaction:				Escherichia coli	00013	
Indole reagent (Vracko and Sherris reagent) ISO 11866-2					00090	
(Vracko and Sherris reagent) ISO 11866-2 formation on membrane filters Citrobacter freundii Salmonella Typhimurium ^c O00031 No pink colour development Salmonella Enteritidis ^c O0024 Positive reaction: Presence of fluorescence under UV radiation (360 ± 20) nm Escherichia coli No pink colour development O0024 Presence of fluorescence under UV radiation (360 ± 20) nm Negative reaction: Presence of fluorescence under UV radiation (360 ± 20) nm					00179	
Sherris reagent) Megative reaction: Salmonella Typhimuriumc O0006 Negative reaction: No pink colour development		ISO 11866-2		Enterobacter aerogenes	00175	
Salmonella Enteritidisc 00031 Salmonella Enteritidisc 00030 Pseudomonas aeruginosa 00024 Presence of fluorescence under 00025 UV radiation (360 ± 20) nm Escherichia coli Negative reaction: Negative reaction:			membrane filters	Citrobacter freundii	00006	Negative reaction:
King's B medium ISO 16266 Detection of fluorescein Detection of fluorescein Detection of fluorescein Escherichia coli Detection of fluorescein Negative reaction: Presence of fluorescence under UV radiation (360 ± 20) nm Negative reaction:				Salmonella Typhimurium ^c	00031	No pink colour development
Pseudomonas aeruginosa 00025 Presence of fluorescence under UV radiation (360 ± 20) nm				Salmonella Enteritidis ^c	00030	
King's B medium ISO 16266 Detection of fluorescein Pseudomonas aeruginosa 00025					00024	Positive reaction:
King's B medium ISO 16266 Detection of fluorescein 00026 UV radiation (360 ± 20) nm 00012 Escherichia coli 00013 Negative reaction:				Pseudomonas aeruginosa	00025	
Ring's B medium ISO 16266 fluorescein					00026	
Escherichia coli 00013 Negative reaction:	King's B medium	ISO 16266			00012	
			fluorescein	Escherichia coli	00013	Negative reaction:
100 Tradicacence					00090	No fluorescence
00179					00179	

^a Strain free of choice; one of the strains has to be used as a minimum.

 $^{^{}b} \qquad \text{Refer to the reference strain catalogue available on } \underline{\text{http://www.wfcc.info}} \text{ for information on culture collection strain numbers and contact details}^{[20]}.$

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

d Weak coagulase-producing strain of S. aureus.

Table K.1 (continued)

Medium/reagent	International Standard	Function	Control strains ^a	WDCM ^b numbers	Characteristic reactions
				00012	
				00013	
			Escherichia coli	00090	Positive reaction:
				00179	The colony material becomes
КОН				00156	stringy — Gram negative
(3 % potassium	ISO 17995	Gram typing	Campylobacter jejuni		organism
hydroxide solution)				00005	
			Campylobacter coli	00004	
					Negative reaction:
			Staphylococcus aureus	00032	Colony material remains smooth — Gram positive organism
					Positive reaction:
Lactose-gelatin	ISO 7937	Detection of gas formation, acid	Clostridium perfringens	00007	Presence of gas, yellow colour and gelatin liquefaction
medium	130 7 937	formation and gelatin liquefaction			Negative reaction:
		nquoruon	Hafnia alvei	00095	No colour change or red, no gelatin liquefaction
	ISO 7937	Detection of lactose fermentation and sulfite reduction			Positive reaction:
			Clostridium perfringens	00007	Gas production: Durham tube more than one quarter full of gas
LS (lactose sulfite) medium					Iron sulfite production: Formation of black precipitate
			Clostridium sporogenes	00008	Negative reaction:
			growth tanam spor egenes		Gas production but no blackening
			Salmonella Typhimurium ^c	00031	
			Salmonella Enteritidis ^c	00030	Positive reaction:
			Enterobacter aerogenes	00175	Medium remains purple after incubation and is turbid
			Vibrio parahaemolyticus	00185	
Lysine	ISO 6579-1		Proteus mirabilis	00023	
decarboxylase medium Lysine	ISO 21567 ISO 19250	Detection of L-Lysine decarboxylase (LDC)	Citrobacter freundii	0006	
decarboxylase saline medium	ISO 21872-1 ISO 22964		Cronobacter sakazakii	00214	Negative reaction:
			Cronobacter muytjensis	00213	Medium changes from purple to yellow
				00012	
			Escherichia coli	00013	
			Escherichia coli	00090	
				00179	
Malaskii		Detection of spores	Bacillus cereus	00001	Positive reaction:
Malachite green oxalate solution	ISO 21871	by microscopic	Ducinus cereus	00001	Green stained spores
		examination	None	_	No green-stained spores

^a Strain free of choice; one of the strains has to be used as a minimum.

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

Weak coagulase-producing strain of S. aureus.

Table K.1 (continued)

Medium/reagent	International Standard	Function	Control strains ^a	WDCM ^b numbers	Characteristic reactions
			Listeria monocytogenes 4b	00021	Positive reaction:
Motility media Non-selective liquid medium including	ISO 11290-1 ISO 11290-2	Detection of motility	Listeria monocytogenes 1/2a	00109	Tumbling motility in liquid medium at 25°C under phase-contrast microscope
sterile water	130 11270 2		Listeria innocua	00017	
			Cil. I.	00032	Negative reaction:
			Staphylococcus aureus	00034	No motility in liquid medium
				00012	
				00013	Positive reaction:
			Escherichia coli	00090 00179	Diffuse growth away from the inoculum line (motile)
Semi-solid nutrient agar for	ISO 11290-1 ISO 11290-2	Detection of motility	Listeria monocytogenes 4b	00021	
motility test			Listeria monocytogenes 1/2a	00109	Umbrella-like growth pattern
			Listeria innocua	00017	
	ISO 21567	Detection of motility			Negative reaction:
			Shigella sonnei	00127	Discrete growth confined to the stab line (non-motile)
			Escherichia coli	00012	D
				00013	Positive reaction:
Mucate broth		Growth in mucate		00090	Growth, medium turns to yellow/ straw colour
(sodium mucate broth)	ISO 21567	broth		00179	
,				00125	Negative reaction:
			Shigella flexneri	00126	No growth, no colour change of the medium (blue colour)
			Lactobacillus casei	00100	Positive reaction:
		Growth at 15 °C	Lactobacillus plantarum	00104	Turbidity
			Lactobacillus delbrueckii	00102	Negative reaction:
MRS broth	ISO 9232		subsp. bulgaricus	30102	No turbidity
			Lactobacillus delbrueckii	00102	Positive reaction:
		Growth at 45 °C	subsp. bulgaricus	00102	Turbidity
		diowinat TJ C	Lactobacillus plantarum	00104	Negative reaction:
			Buccobucillus piuntui um	30101	No turbidity

^a Strain free of choice; one of the strains has to be used as a minimum.

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

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

 $^{^{}m d}$ Weak coagulase-producing strain of S. aureus.

Table K.1 (continued)

Medium/reagent	International Standard	Function	Control strains ^a	WDCM ^b numbers	Characteristic reactions
			Escherichia coli Clostridium perfringens	00012 00013 00090 00179	Motility: Positive reaction: Diffuse growth out into the medium away from the stab line Reduction of nitrate to nitrite: Positive reaction:
Nitrate motility	ISO 7937	Detection of motility and reduction of	closti lulum perji mgens	00007	Red colour after adding the nitrite detection reagents or no colour after addition of zinc dust
mearum		nitrate to nitrite		00007	Motility: Negative reaction:
			Clostridium perfringens	00007	Discrete line of growth along the stab line without diffusion into the medium
					Reduction of nitrate to nitrite:
			Clostridium sporogenes	00008	Negative reaction:
			Clostridium bifermentans	00079	No red colour after adding the nitrite detection reagents; red colour after addition of zinc dust
			Proteus mirabilis	00023	
			Enterobacter aerogenes	00175	Positive reaction:
			Cronobacter sakazakii	00214	Turbidity and purple (violet) colour
Ornithine decarboxylase	ISO 21567	Detection of	Cronobacter muytjensis	00213	
medium Ornithine decarboxylase saline medium	ISO 22964	L-Ornithine	Shigella sonnei	00127	
	130 22 904	decarboxylase (ODC)	Citrobacter freundii	00006	
			Pseudomonas aeruginosa	00024 00025 00026	Negative reaction: Yellow colour
			Pseudomonas fluorescens	00115	

^a Strain free of choice; one of the strains has to be used as a minimum.

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 can require the use of a different serovar. Refer to national requirements relating to the choice of *Salmonella* serovars.

 $^{^{}m d}$ Weak coagulase-producing strain of S. aureus.

Table K.1 (continued)

Medium/reagent	International Standard	Function	Control strains ^a	WDCM ^b numbers	Characteristic reactions
				00024	
			Pseudomonas aeruginosa	00025	
	ISO 9308-1			00026	Positive reaction:
	ISO 21528-1		Pseudomonas fluorescens	00115	Mauve, violet, purple or dark blue colour in the reaction time
	ISO 21528-2		1 seudomonas juorescens	00113	
	ISO 21872-1		Vibrio parahaemolyticus	00185	
	ISO 22964	Detection of		00012	
Oxidase reagent	ISO/TS 11059	cytochrome oxidase		00013	
	ISO 13720		Escherichia coli	00090	
	ISO 13722			00179	Negative reaction:
	ISO 16266 ISO 17995		Cronobacter sakazakii	00214	No colour change in the reaction time
	130 17 9 9 3		Cronobacter muytjensis	00213	
			Brochothrix thermosphacta	00071	
	ISO 21872-1		0 % NaCl Vibrio cholerae non O1/ non O139	00203	
				00037	
			6 % NaCl Vibrio parahaemolyticus	00138	
				00185	Positive reaction:
			Vibrio vulnificus	00139	Growth (turbidity)
Peptone waters			10 % NaCl Staphylococcus aureus	00032 00034	
with different NaCl		Detection of	0 % NaCl	00185	
concentration:		halotolerance	Vibrio parahaemolyticus	00100	
0 %, 6 %, 10 %			6 % NaCl Vibrio cholerae non 01/ non 0139	00203	
			10 % NaCl Vibrio cholerae non O1/	00203	Negative reaction:
			non 0139	00037	No growth (no turbidity)
			Vibrio parahaemolyticus	00138	
				00185	
			Vibrio vulnificus	00139	
				00032	Positive reaction:
			Staphylococcus aureus	00034	Clotting of the plasma; volume of
				00035d	the clot occupies more than the half of the liquid
Rabbit plasma	ISO 6888-1	Detection of coagulase	Staphylococcus epidermidis	00036	Negative reaction:
			Staphylococcus saprophyticus	00159	No sign of clotting of the plasma while control plasma shows no clotting with sterile Brain Heart infusion broth

^a Strain free of choice; one of the strains has to be used as a minimum.

Refer to the reference strain catalogue available on $\frac{\text{http://www.wfcc.info}}{\text{for information on culture collection strain numbers and contact details}} [20].$

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

Weak coagulase-producing strain of *S. aureus*.

Table K.1 (continued)

Medium/reagent	International Standard	Function	Control strains ^a	WDCM ^b numbers	Characteristic reactions
Safranin solution	ISO 21871	Detection of sporangia by microscopic examination	Bacillus cereus	00001	Positive reaction: Red staining of sporangia
			None	_	No stained sporangia
with toluone and	ISO 6579-1 ISO 21567	Detection of β-Galactosidase	Escherichia coli	00012 00013 00090 00179	Positive reaction: Yellow colour
reagent	ISO 21872-1		Proteus mirabilis	00023	Negative reaction:
			Vibrio parahaemolyticus	00185	No colour change
	ISO 7932 ISO 21871		Bacillus cereus Listeria monocytogenes	00001 00021 00109	Positive reaction: Zone of β-haemolysis; colony surrounded by a clear zone
Sheep blood agar for haemolysis test		Detection of β-haemolysis	Bacillus subtilis subsp.	00109	
	ISO 11290-1 ISO 11290-2		Bacillus subtilis	00070	Negative reaction: No zone of haemolysis
			Listeria innocua	00017	
Sudan black B	ISO 21871	Detection of intra- cellular fat by microscopic examination	Bacillus cereus	00001	Positive reaction: Black staining of intracellular fat in the cells
			Bacillus subtilis	00070	No stained intracellular fat in the cells

^a Strain free of choice; one of the strains has to be used as a minimum.

 $^{^{}b} \qquad \text{Refer to the reference strain catalogue available on } \underline{\text{http://www.wfcc.info}} \text{ for information on culture collection strain numbers and contact details}^{[20]}.$

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

 $^{^{\}rm d} \qquad \text{Weak coagulase-producing strain of } \textit{S. aureus}.$

Table K.1 (continued)

Medium/reagent	International Standard	Function	Control strains ^a	WDCM ^b numbers	Characteristic reactions
					Characteristic reactions see specific standard
					Butt:
					Yellow: Glucose fermented
			Salmonella Enteritidis ^c Salmonella Typhimurium ^c	00030	Black: Formation of hydrogen sulphite
					Bubbles or cracks: Gas formation
					Slant surface:
		Multiple function			Yellow: Lactose and/or sucrose utilized
	ISO 6579-1	Fermentation of			Butt:
Triple sugar iron	ISO 19250	glucose, lactose, sucrose with or			Yellow: Glucose fermented
agar (TSI)	ISO 21567	without gas	CI · II · ·	00405	No gas formation
		Detection of H ₂ S formation	Shigella sonnei Shigella flexneri	00127 00125	No formation of hydrogen sulphite
					Slant surface:
					Red or unchanged: Lactose and sucrose not utilized
			Pseudomonas aeruginosa	00025	Butt:
					Red or unchanged: Glucose not fermented
					Slant surface:
					Red or unchanged: Lactose and sucrose not utilized
			Proteus mirabilis	0023	Positive reaction:
			Klebsiella pneumoniae	00097	Liberation of ammonia with colour change to rose/rose-pink/deep cerise
				00012	
	100 045 65			00013	
	ISO 21567	Detection of urea	Escherichia coli	00090	
Urea agar (Christensen)	ISO 6579-1	hydrolysis by ammonia		00179	
	ISO 19250	production	G 1	00001	Negative reaction:
	ISO 10273		Salmonella Typhimurium ^c	00031	No liberation of ammonia, no change of colour
			Salmonella Enteritidis ^c	00030	change of colour
			Shigella sonnei	00127	
			Shigella flexneri	00125	

^a Strain free of choice; one of the strains has to be used as a minimum.

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

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

d Weak coagulase-producing strain of S. aureus.

Table K.1 (continued)

Medium/reagent	International Standard	Function	Control strains ^a	WDCM ^b numbers	Characteristic reactions
	ISO 11290-1 ISO 11290-2	Detection of acetylmethylcarbinol from glucose fermentation	Listeria monocytogenes 4b	00021	Positive reaction:
Voges-			Listeria monocytogenes 1/2a	00109	Formation of a pink to bright red layer within 15 min of adding the required reagents and shaking
Proskauer medium with			Listeria innocua	00017	
reagents				00012	
			Escherichia coli	00013	Negative reaction:
				00090	No formation of a pink to bright red layer
				00179	

^a Strain free of choice; one of the strains has to be used as a minimum.

Bibliography

Replace the following references (which have been revised):

- [59] ISO 10272-2, Microbiology of the food chain Horizontal method for detection and enumeration of Campylobacter spp. Part 2: Colony-count technique
- [83] ISO 21872-1, 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

Add the following references (cited in Table K.1):

- [88] ISO 9232, *Yogurt Identification of characteristic microorganisms* (Lactobacillus delbrueckii *subsp.* bulgaricus *and* Streptococcus thermophilus)
- [89] ISO 11866-1, Milk and milk products Enumeration of presumptive Escherichia coli Part 1: Most probable number technique using 4-methylumbelliferyl-β-D-glucuronide (MUG)
- [90] ISO 11866-2, Milk and milk products Enumeration of presumptive Escherichia coli Part 2: Colony-count technique at 44 °C using membranes
- [91] ISO 13722, Microbiology of the food chain Enumeration of Brochothrix spp. Colony-count technique
- [92] ISO 21567, Microbiology of food and animal feeding stuffs Horizontal method for the detection of Shigella spp.
- [93] ISO 22964, *Microbiology of the food chain Horizontal method for the detection of* Cronobacter *spp.*

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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 can require the use of a different serovar. Refer to national requirements relating to the choice of *Salmonella* serovars.

d Weak coagulase-producing strain of S. aureus.