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AMENDMENT 2
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Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media

AMENDMENT 2

Microbiologie des aliments, des aliments pour animaux et de l'eau — Préparation, production, stockage et essais de performance des milieux de culture

AMENDEMENT 2

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*, in collaboration with Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 4, *Microbiological methods*, and 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).

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

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 Table K.1 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 Table K.1 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 Table K.1. 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
Acetamide broth with Nessler's reagent	ISO 16266	Detection of ammonia production from acetamide	*Pseudomonas aeruginosa*	00024 00025 00026	Positive reaction: Yellow to brick red after adding 1 to 2 drops of Nessler's reagent
			Escherichia coli	00012 00013 00090 00179	Negative reaction: No yellow to brick red colour
Acetate agar (Sodium acetate agar)	ISO 21567	Growth on acetate agar	*Escherichia coli*	00012 00013 00090 00179	Positive reaction: Blue colonies with surrounding medium blue/green
			Shigella sonnei *Shigella flexneri*	00127 00125	Negative reaction: No growth or very weak growth, no colour change of the medium (remains green)
Acid phosphatase reagent	ISO 14189	Detection of acid phosphatase	*Clostridium perfringens*	00007 00080 00174	Positive reaction: Mauve/purple/violet colour
			Clostridium bifermentans	00079	Negative reaction: No mauve/purple/violet colour
Arginine dihydrolase saline medium	ISO 21872-1	Detection of L-Arginine dihydrolase	*Vibrio fluvialis*	00137	Positive reaction: Turbidity and violet/purple colour
			Vibrio parahaemolyticus	00037 00185	Negative reaction: Yellow colour
Bile aesculin azide agar	ISO 7899-2	Detection of aesculin hydrolysis	*Enterococcus faecalis* *Enterococcus faecium*	00009 00087 00176 00177 00178	Positive reaction: Tan to black colour in the surrounding medium
			Aerococcus viridans *Escherichia coli*	00061 00012 00013 00090 00179	Negative reaction: No tan to black colour in the surrounding medium

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

[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*.

Table K.1 *(continued)*

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

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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*.

Table K.1 *(continued)*

Medium/reagent	International Standard	Function	Control strains[a]	WDCM[b] numbers	Characteristic reactions
Catalase reagent (3 % hydrogen peroxide solution)	ISO 9232 ISO 10272-1 ISO 10272-2 ISO 11290-1 ISO 11290-2	Detection of catalase after adding hydrogen peroxide solution	*Staphylococcus aureus*	00032 00034	Positive reaction: Formation of bubbles of oxygen
			Campylobacter jejuni	00005	
			Listeria monocytogenes	00020 00021	
			Listeria innocua	00017	
			Listeria ivanovii	00018	
			Enterococcus faecalis	00009 00087 00176	Negative reaction: No formation of bubbles of oxygen
			Enterococcus faecium	00177 00178	
			Lactobacillus delbrueckii subsp. *bulgaricus*	00121	
Citrate agar (Christensen's citrate agar)	ISO 21567	Growth on citrate agar	*Enterobacter aerogenes*	00175	Positive reaction: Cream/pink growth with surrounding medium red
			Enterobacter cloacae	00083	
			Shigella sonnei	00127	Negative reaction: No growth
			Shigella flexneri	00125 00126	
Glucose agar O-F medium with overlay	ISO/TS 11059 ISO 21528-1 ISO 21528-2	Production of acid from glucose	*Escherichia coli*	00012 00013 00090 00179	Positive reaction: Yellow colour
			Pseudomonas aeruginosa	00024 00025 00026	Negative reaction: Growth, but no yellow colour development
			Pseudomonas fluorescens	00115	
Glucose MRS broth with overlay agar	ISO 9232	Detection of CO_2 production	*Lactobacillus brevis*	00099	Positive reaction: Agar layer detaches itself from the underlying contents
			Lactobacillus delbrueckii subsp. *bulgaricus*	00102	Negative reaction: No gas production, agar layer not detached

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

[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*.

Table K.1 *(continued)*

Medium/reagent	International Standard	Function	Control strains[a]	WDCM[b] numbers	Characteristic reactions
Indole reaction medium/reagent Tryptone/ tryptophan medium with indole reagent (Kovacs reagent) Tryptone/ tryptophan saline medium with indole reagent (Kovacs reagent) Indole reagent (Kovacs reagent)	ISO 6579-1 ISO 9308-1 ISO 16654 ISO 21567 ISO 21872-1 ISO 11866-1	Detection of indole formation from tryptophan	*Escherichia coli*	00012 00013 00090 00179	
			Vibrio parahaemolyticus	00037 00138 00185	Positive reaction: Formation of a red ring within 10 min
			Vibrio cholerae	00203	
			Vibrio vulnificus	00139	
			Enterobacter aerogenes	00175	
			Citrobacter freundii	00006	Negative reaction: Yellow/brown ring within 10 min
			Salmonella Typhimurium[c]	00031	
			Salmonella Enteritidis[c]	00030	
Indole reagent (Vracko and Sherris reagent)	ISO 11866-2	Detection of indole formation on membrane filters	*Escherichia coli*	00012 00013 00090 00179	Positive reaction: Development of a pink colour within a few minutes
			Enterobacter aerogenes	00175	
			Citrobacter freundii	00006	Negative reaction: No pink colour development
			Salmonella Typhimurium[c]	00031	
			Salmonella Enteritidis[c]	00030	
King's B medium	ISO 16266	Detection of fluorescein	*Pseudomonas aeruginosa*	00024 00025 00026	Positive reaction: Presence of fluorescence under UV radiation (360 ± 20) nm
			Escherichia coli	00012 00013 00090 00179	Negative reaction: No fluorescence

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

[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*.

Table K.1 *(continued)*

Medium/reagent	International Standard	Function	Control strains[a]	WDCM[b] numbers	Characteristic reactions
KOH (3 % potassium hydroxide solution)	ISO 17995	Gram typing	*Escherichia coli*	00012 00013 00090 00179	Positive reaction: The colony material becomes stringy — Gram negative organism
			Campylobacter jejuni	00156 00005	
			Campylobacter coli	00004	
			Staphylococcus aureus	00032 00034	Negative reaction: Colony material remains smooth — Gram positive organism
Lactose-gelatin medium	ISO 7937	Detection of gas formation, acid formation and gelatin liquefaction	*Clostridium perfringens*	00007	Positive reaction: Presence of gas, yellow colour and gelatin liquefaction
			Hafnia alvei	00095	Negative reaction: No colour change or red, no gelatin liquefaction
LS (lactose sulfite) medium	ISO 7937	Detection of lactose fermentation and sulfite reduction	*Clostridium perfringens*	00007	Positive reaction: Gas production: Durham tube more than one quarter full of gas Iron sulfite production: Formation of black precipitate
			Clostridium sporogenes	00008	Negative reaction: Gas production but no blackening
Lysine decarboxylase medium Lysine decarboxylase saline medium	ISO 6579-1 ISO 21567 ISO 19250 ISO 21872-1 ISO 22964	Detection of L-Lysine decarboxylase (LDC)	*Salmonella* Typhimurium[c]	00031	Positive reaction: Medium remains purple after incubation and is turbid
			Salmonella Enteritidis[c]	00030	
			Enterobacter aerogenes	00175	
			Vibrio parahaemolyticus	00185	
			Proteus mirabilis	00023	Negative reaction: Medium changes from purple to yellow
			Citrobacter freundii	0006	
			Cronobacter sakazakii	00214	
			Cronobacter muytjensis	00213	
			Escherichia coli	00012 00013 00090 00179	
Malachite green oxalate solution	ISO 21871	Detection of spores by microscopic examination	*Bacillus cereus*	00001	Positive reaction: Green stained spores
			None	—	No green-stained spores

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

[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*.

Table K.1 *(continued)*

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

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

[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*.

Table K.1 *(continued)*

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

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

[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*.

Table K.1 *(continued)*

Medium/reagent	International Standard	Function	Control strains[a]	WDCM[b] numbers	Characteristic reactions
Oxidase reagent	ISO 9308-1 ISO 21528-1 ISO 21528-2 ISO 21872-1 ISO 22964 ISO/TS 11059 ISO 13720 ISO 13722 ISO 16266 ISO 17995	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 %	ISO 21872-1	Detection of halotolerance	0 % NaCl *Vibrio cholerae* non O1/ non O139	00203	Positive reaction: Growth (turbidity)
			6 % NaCl *Vibrio parahaemolyticus*	00037 00138 00185	
			Vibrio vulnificus	00139	
			10 % NaCl *Staphylococcus aureus*	00032 00034	
			0 % NaCl *Vibrio parahaemolyticus*	00185	Negative reaction: No growth (no turbidity)
			6 % NaCl *Vibrio cholerae* non O1/ non O139	00203	
			10 % NaCl *Vibrio cholerae* non O1/ non O139	00203	
			Vibrio parahaemolyticus	00037 00138 00185	
			Vibrio vulnificus	00139	
Rabbit plasma	ISO 6888-1	Detection of coagulase	*Staphylococcus aureus*	00032 00034 00035[d]	Positive reaction: Clotting of the plasma; volume of the clot occupies more than the half of the liquid
			Staphylococcus epidermidis	00036	Negative reaction: No sign of clotting of the plasma while control plasma shows no clotting with sterile Brain Heart infusion broth
			Staphylococcus saprophyticus	00159	

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[d] Weak coagulase-producing strain of *S. aureus*.