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Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Escherichia coli* O157

AMENDMENT 2: Inclusion of performance testing of all culture media and reagents

Microbiologie des aliments — Méthode horizontale pour la recherche des Escherichia coli O157

AMENDMENT 2: Inclusion d'essais de performance de tous les 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 food and animal feeding stuffs — Horizontal method for the detection of *Escherichia coli* O157

AMENDMENT 2: Inclusion of performance testing of all culture media and reagents

Clause 2

Add the following normative reference:

ISO 11133, *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*

Clause 5, first sentence

Replace the text with the following:

For current laboratory practice, see ISO 7218 and ISO 11133.

The general specifications of ISO 11133 are applicable to the preparation and performance testing of the culture media and reagents described in this clause. If culture media or reagents are prepared from dehydrated complete media/reagents or if ready-to-use media/reagents are used, follow the manufacturer's instructions regarding preparation, storage conditions, expiry date and use.

The shelf lives of the media and reagents indicated in this clause have been determined in previous studies. The user shall verify these under their own storage conditions (in accordance with ISO 11133).

Performance testing of culture media and reagents is described in 5.11.

Clause 5, after 5.10

Add the following text as a new subclause.

5.11 Performance testing

The definitions of productivity, selectivity and specificity are specified in ISO 11133. For performance testing of culture media, follow the procedures in accordance with ISO 11133 and Table 1.

Table 1 — Performance testing for the quality assurance of the culture media and reagents

Medium	Function	Incubation	Control strains	WDCM numbers[a]	Method of control	Criteria[b]
mTSB + N[c]	Productivity	6 h/ (41,5 ± 1) °C	*Escherichia coli* O157:H7 + *Staphylococcus aureus* subsp. *aureus*[e]	00014 (non-toxigenic strain) 00032 or 00034	Qualitative	> 10 characteristic colonies on CT-SMAC or other medium of choice (characteristic appearance of the colonies should refer to the different media used)
	Selectivity	(21 ± 3) h/ (41,5 ± 1) °C	*Staphylococcus aureus* subsp. *aureus*[e]	00032 or 00034	Qualitative	Total inhibition (0) on TSA
CT-SMAC[c]	Productivity	(21 ± 3) h/ (37 ± 1) °C	*Escherichia coli* O157:H7	00014 (non-toxigenic strain)	Qualitative	Good growth (2) of transparent colonies with a pale yellowish-brown appearance and a diameter ~1 mm
	Selectivity		*Staphylococcus aureus* subsp. *aureus*[d]	00032 or 00034	Qualitative	Total inhibition (0)
			Escherichia coli[d]	00012 or 00013	Qualitative	Partial inhibition (1) with growth of some pink colonies
Nutrient agar	Productivity	(21 ± 3) h/ (37 ± 1) °C	*Escherichia coli*[d]	00012 or 00013 or 00014 (non-toxigenic strain)	Qualitative	Good growth (2)
Tryptone / tryptophan medium with indole reagent (Kovac's reagent)	Detection of indole formation from tryptophan	(21 ± 3) h/ (37 ± 1) °C, then Kovac's reagent is added and allow to stand at room temperature for 10 min	*Escherichia coli*[d]	00012 00013 00014 (non-toxigenic strain) 00090 00179	Qualitative	Positive reaction: Formation of a red ring within 10 min
		(21 ± 3) h/ (37 ± 1) °C	*Klebsiella aerogenes* (formerly *Enterobacter aerogenes*)[d] *Citrobacter freundii*[d] *Salmonella enterica* serovar Typhimurium[d,e] *Salmonella enterica* serovar Enteritidis[d,e]	00175 00006 00031 00030	Qualitative	Negative reaction: Yellow/brown ring within 10 min

[a] Refer to the reference strain catalogue on http://www.wfcc.info for information on culture collection strain numbers and contact details; WDCM: World Data Centre for Microorganisms.

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

[c] mTSB +N: Modified Tryptone soya broth supplemented with 20 mg/l of novobiocin; CT-SMAC: Cefixime tellurite sorbitol MacConkey agar.

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

[e] Some national restrictions and directions require the use of a different serovar. Make reference to national requirements relating to the choice of *Salmonella* serovar(s).