
**Microbiology of food and animal feeding
stuffs — Guidelines on preparation and
production of culture media —**

Part 2:

**Practical guidelines on performance
testing of culture media**

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**AMENDMENT 1: Test microorganisms for
commonly used culture media**

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*Microbiologie des aliments — Guide pour la préparation et la production
des milieux de culture —*

*Partie 2: Guide général pour les essais de performance des milieux de
culture*

*AMENDEMENT 1: Micro-organismes pour essai recommandés pour les
milieux de culture les plus usuels*



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Amendment 1 to ISO/TS 11133-2:2003 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 9, *Microbiology*.

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Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media —

Part 2

Practical guidelines on performance testing of culture media

AMENDMENT 1: Test microorganisms for commonly used culture media

Page 1, Clause 2

Add the following entries:

ISO 4831, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of coliforms — Most probable number technique*

ISO 4832, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique*

ISO 4833, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of microorganisms — Colony-count technique at 30 °C*

ISO 6579, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Salmonella spp.*

ISO 6887-1, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions*

ISO 6887-2, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 2: Specific rules for the preparation of meat and meat products*

ISO 6887-3, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 3: Specific rules for the preparation of fish and fishery products*

ISO 6887-4, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 4: Specific rules for the preparation of products other than milk and milk products, meat and meat products, and fish and fishery products*

ISO 6887-5, *Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 5: Specific rules for the preparation of milk and milk products*

ISO 6888-1, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 1: Technique using Baird-Parker agar medium*

ISO 6888-2, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 2: Technique using rabbit plasma fibrinogen agar medium*

ISO 6888-3, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 3: Detection and MPN technique for low numbers*

ISO 7251, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive Escherichia coli — Most probable number technique*

ISO 7932, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of presumptive Bacillus cereus — Colony-count technique at 30 °C*

ISO 7937, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of Clostridium perfringens — Colony-count technique*

ISO 10272-1, *Microbiology of food and animal feeding stuffs — Horizontal method for detection and enumeration of Campylobacter spp. — Part 1: Detection method*

ISO/TS 10272-2, *Microbiology of food and animal feeding stuffs — Horizontal method for detection and enumeration of Campylobacter spp. — Part 2: Colony count technique*

ISO/TS 10272-3, *Microbiology of food and animal feeding stuffs — Horizontal method for detection and enumeration of Campylobacter spp. — Part 3: Semi-quantitative method*

ISO 10273, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of presumptive pathogenic Yersinia enterocolitica*

ISO 11290-1, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of Listeria monocytogenes — Part 1: Detection method*

ISO 11290-2, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of Listeria monocytogenes — Part 2: Enumeration method*

ISO 15213, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions*

ISO 15214, *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of mesophilic lactic acid bacteria — Colony-count technique at 30° C*

ISO 16649 (all parts), *Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli*

ISO 16654, *Microbiology of food and animal feeding stuffs — Horizontal method for the detection of Escherichia coli O157*

ISO 21528-1, *Microbiology of food and animal feeding stuffs — Horizontal methods for the detection and enumeration of Enterobacteriaceae — Part 1: Detection and enumeration by MPN technique with pre-enrichment*

ISO 21528-2, *Microbiology of food and animal feeding stuffs — Horizontal methods for the detection and enumeration of Enterobacteriaceae — Part 2: Colony-count method*

Replace the whole of Annex B by the following text.

Annex B (normative)

Test microorganisms for commonly used culture media (giving information on the culture medium, culture conditions, test microorganisms, culture collection number of test organisms and the expected reactions)

Tables B.1 to B.6 have been established taking into account the control strains used in the European Pharmacopoeia (EP) and the recommendations from the EP on food microbiology for culture media [Working Party of the International Committee on Food Microbiology and Hygiene (ICFMH)]. These criteria shall be included in specific International Standards when prepared or revised in the future. A validated batch of media is one which has shown satisfactory performance. The strain numbers specified in Tables B.1 to B.7 are those from the catalogue of universal strain identifiers, containing details of the reference strains represented by each WDCM number and contact details of the culture collections, compiled by the World Data Centre for Microorganisms (WDCM) (see Reference [16]). All cited media are described within International Standards.

If strain variability is encountered, investigate the effect of the culture medium (e.g. by obtaining the same medium from a different manufacturer) and obtain a further reference culture from the culture collection in which it was originally deposited. Users are requested to feed relevant information on strain variability back to WG 5, *Culture media*, of ISO/TC 34/SC 9 through the secretariat of ISO/TC 34/SC 9.

Information on performance testing already published in International Standards has not been included in Tables B.1 to B.7.

Table B.1 — Selective media for enumeration of microorganisms

Medium	Type	Microorganisms	Standard	Function	Incubation	Control strains (see Reference [16])	Reference medium	Method of control	Criteria	Characteristic reactions
Baird - Parker	S ^a	Coagulase-positive staphylococci	ISO 6888-1	Productivity	24 h to 48 h at 37 °C	<i>Staphylococcus aureus</i> WDCM 00032 <i>Staph. aureus</i> WDCM 00034 ^b	TSA	Quantitative	Minimum PR ^c value: 0,5	Black or grey colonies with clear halo (egg yolk clearing reaction)
				Selectivity	48 h at 37 °C	<i>E. coli</i> WDCM 00013 or WDCM 00012 ^b		Qualitative	Total inhibition	
				Specificity	24 h to 48 h at 37 °C	<i>Staphylococcus epidermidis</i> WDCM 00036 or <i>S. saprophyticus</i> WDCM 00159 ^b		Qualitative		Black or grey colonies without egg yolk clearing reaction
RPFA	S	Coagulase-positive staphylococci	ISO 6888-2	Productivity	24 h to 48 h at 37 °C	<i>Staph. aureus</i> WDCM 00032 <i>Staph. aureus</i> WDCM 00034 ^b	TSA	Quantitative	Minimum PR ^c value: 0,5	Black or grey colonies with opacity halo
				Selectivity	48 h at 37 °C	<i>E. coli</i> WDCM 00013 or WDCM 00012 ^b		Qualitative	Total inhibition	
				Specificity	24 h to 48 h at 37 °C	<i>Staphylococcus epidermidis</i> WDCM 00036 or <i>S. saprophyticus</i> WDCM 00159 ^b		Qualitative		Black or grey colonies without opacity halo
MRS	S	Lactic acid bacteria	ISO 15214	Productivity	72 h at 30 °C	<i>Lactobacillus sakei</i> WDCM 00015 ^b <i>Pediococcus pentosaceus</i> WDCM 00158 <i>Lactococcus lactis</i> WDCM 00016 ^b	Media batch MRS already validated	Quantitative	Minimum PR ^c value: 0,7	Characteristic colonies according to each species
				Selectivity	72 h at 30 °C	<i>E. coli</i> WDCM 00013 or WDCM 00012 ^b		Qualitative	Total inhibition	
				Specificity	24 h to 48 h at 37 °C	<i>Bacillus cereus</i> WDCM 00001		Qualitative		Black or grey colonies without opacity halo
MYP	S	<i>Bacillus cereus</i>	ISO 7932	Productivity	24 h to 48 h at 30 °C	<i>B. cereus</i> WDCM 00001 ^b	TSA	Quantitative	Minimum PR ^c value: 0,5	Pink colonies with precipitation halo
				Selectivity	48 h at 30 °C	<i>E. coli</i> WDCM 00013 or WDCM 00012 ^b		Qualitative	Total inhibition	
				Specificity	48 h at 30 °C	<i>Bacillus subtilis</i> WDCM 00003 ^b		Qualitative		Yellow colonies without precipitation halo
Agar <i>Listeria</i> according to Ottaviani and Agosti	S	<i>Listeria monocytogenes</i>	ISO 11290 (all parts)	Productivity	48 h at 37 °C	<i>L. monocytogenes</i> 1/2a WDCM 00020 <i>L. monocytogenes</i> 4b WDCM 00021 ^b	TSA	Quantitative	Minimum PR ^c value: 0,5	Blue green colonies with opaque halo
				Selectivity	48 h at 37 °C	<i>E. coli</i> WDCM 00013 or WDCM 00012 ^b <i>Enterococcus faecalis</i> WDCM 00087 or <i>E. faecalis</i> WDCM 00009 ^b		Qualitative	Total inhibition	
				Specificity	48 h at 37 °C	<i>Listeria innocua</i> WDCM 00017		Qualitative		Blue green colonies without opaque halo

Table B.1 (continued)

Medium	Type	Microorganisms	Standard	Function	Incubation	Control strains (see Reference [16])	Reference medium	Method of control	Criteria	Characteristic reactions
TS(C)	S	<i>Clostridium perfringens</i>	ISO 7937	Productivity	20 h at 37 °C anaerobic atm.	<i>C. perfringens</i> WDCM 00007 ^b <i>C. perfringens</i> WDCM 00080	Media batch TS(C) already validated	Quantitative	Minimum PR ^c value: 0,7	Black colonies
				Selectivity TSC	20 h at 37 °C anaerobic atm.	<i>E. coli</i> WDCM 00013 or WDCM 00012 ^b		Qualitative	Total inhibition	
TS	S	Sulfite-reducing bacteria	ISO 15213	Productivity	24 h to 48 h at 37 °C anaerobic atm.	<i>C. perfringens</i> WDCM 00007 ^b <i>C. perfringens</i> WDCM 00080	Media batch TS already validated	Quantitative	Minimum PR ^c value: 0,7	Black colonies
				Selectivity TS		<i>E. coli</i> WDCM 00013 or WDCM 00012 ^b		Qualitative	—	White colonies
VRBG	S	Enterobacteriaceae	ISO 21528 (all parts)	Productivity	24 h at 37 °C	<i>E. coli</i> WDCM 00013 or WDCM 00012 ^b <i>Salmonella</i> Typhimurium WDCM 00081	TSA	Quantitative	Minimum PR ^c value: 0,5	Pink to red colonies with or without precipitation halo
				Selectivity	24 h at 37 °C	<i>E. faecalis</i> WDCM 00087 or WDCM 00069 ^b		Qualitative	Total inhibition	
VRBL	S	Coliforms	ISO 4832	Productivity	24 h at 30 °C	<i>E. coli</i> WDCM 00013 or WDCM 00012 ^b	TSA	Quantitative	Minimum PR ^c value: 0,5	Purplish-red colonies with or without precipitation halo
				Selectivity	24 h at 30 °C	<i>E. faecalis</i> WDCM 00087 or WDCM 00009 ^b		Qualitative	Total inhibition	
CT-SMAC	S	<i>Escherichia coli</i> O157	ISO 16654	Productivity	24 h at 37 °C	<i>Pseudomonas aeruginosa</i> WDCM 00025 <i>E. coli</i> O 157:H7 WDCM 00014 (non-toxicogenic)	TSA	Quantitative	Minimum PR ^c value: 0,5	Transparent colonies with a pale yellowish-brown appearance and a diameter ~1 mm
				Selectivity	24 h at 37 °C	<i>Staph. aureus</i> WDCM 00032 or WDCM 00034 ^b <i>E. coli</i> WDCM 00090 or WDCM 00013 ^b		Qualitative	Total inhibition	
BGBLB	L ^d	Coliforms	ISO 4831	Productivity	24 h to 48 h at 30 °C	<i>E. coli</i> WDCM 00013 or WDCM 00012 ^b <i>Citrobacter freundii</i> WDCM 00006		Qualitative	Turbidity 2 and gas in Durham tube	Gas production and turbidity
				Selectivity	24 h to 48 h at 30 °C	<i>E. faecalis</i> WDCM 00087 or WDCM 00009 ^b		Qualitative	Partial inhibition without gas production	