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AMENDMENT 1  
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**Yogurt — Identification of  
characteristic microorganisms  
(*Lactobacillus delbrueckii* subsp.  
*bulgaricus* and *Streptococcus*  
*thermophilus*)**

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

*Yaourt — Identification des micro-organismes caractéristiques  
(Lactobacillus delbrueckii subsp. bulgaricus et Streptococcus  
thermophilus)*

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



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This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*, and the International Dairy Federation (IDF). It is being published jointly by ISO and IDF.

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This document was prepared by the IDF *Standing Committee on Analytical Methods for Dairy Microorganisms* and ISO Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 5, *Milk and milk products*. It is being published jointly by ISO and IDF.

The work was carried out by the *Standing Committee on Analytical Methods for Dairy Microorganisms* under the aegis of its project leader Mrs Barbara Gerten (DE).

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# Yogurt — Identification of characteristic microorganisms (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*)

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

### Clause 2

Replace the reference to ISO 8261 | IDF 122 with the following:

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

Add the following normative reference:

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

The titles of ISO 6887-1 and ISO 7218 are updated to their latest versions:

ISO 6887-1, *Microbiology of the food chain — 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 7218, *Microbiology of the food chain — General requirements and guidance for microbiological examinations*

### Clause 5, first paragraph

Replace the reference to “ISO 8261 | IDF 122” with “ISO 6887-5”.

### Clause 5, after the first paragraph

Add the following text:

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 life of the media and reagents indicated in this clause has been determined in some studies. The user shall verify this under its own storage conditions (in accordance with ISO 11133).

Performance testing of culture media and reagents is described in 5.5.

After 5.4

Add the following as 5.5.

### 5.5 Performance testing

The definition of productivity is specified in ISO 11133. In general, follow the procedures for performance testing described in ISO 11133. Table 1 provides the performance testing for the quality assurance of the culture media and reagents.

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

Medium or reagent	Function	Incubation	Control strains <sup>a</sup>	WDCM numbers <sup>b</sup>	Method of control	Criteria <sup>c</sup>		
Catalase reagent (3 % hydrogen peroxide solution)	Detection of catalase after adding hydrogen peroxide solution	Room temperature over 20 min	<i>Bacillus subtilis</i> subsp. <i>spizizenii</i>	00003	Qualitative	Positive reaction: Formation of bubbles of oxygen		
			<i>Staphylococcus aureus</i> subsp. <i>aureus</i>	00032 00034				
			<i>Campylobacter jejuni</i> subsp. <i>jejuni</i>	00005				
			<i>Listeria monocytogenes</i> serovar 1	00020				
			<i>Listeria monocytogenes</i> serovar 4b	00021				
			<i>Listeria innocua</i>	00017				
			<i>Listeria ivanovii</i> subsp. <i>ivanovii</i> serovar 5	00018				
			<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	00102			Qualitative	Negative reaction: No formation of bubbles of oxygen
			<i>Streptococcus thermophilus</i>	00134				
			<i>Enterococcus faecalis</i>	00009 00087 00176				
<i>Enterococcus faecium</i>	00177 00178							

<sup>a</sup> 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).

<sup>b</sup> 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.

<sup>c</sup> Growth is categorized as 0: no growth; 1: weak growth (partial inhibition); 2: good growth (see ISO 11133).

Table 1 (continued)

Medium or reagent	Function	Incubation	Control strains <sup>a</sup>	WDCM numbers <sup>b</sup>	Method of control	Criteria <sup>c</sup>
Glucose MRS broth with overlay agar	Detection of CO <sub>2</sub> production	18 h ± 3 h / 37 °C ± 1 °C	<i>Lactobacillus brevis</i>	00099	Qualitative	Positive reaction: Agar layer detaches itself from the underlying contents
			<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	00102	Qualitative	Negative reaction: No gas production, agar layer not detached
Litmus milk	Growth at 10 °C	7 d / 10 °C ± 1 °C	<i>Streptococcus thermophilus</i>	00134	Qualitative	Negative reaction: No change
	Growth at 45 °C	Up to 7 d / 45 °C ± 1 °C	<i>Streptococcus thermophilus</i>	00134	Qualitative	Positive reaction: Turn to pink and then coagulation. After coagulation, the colour remains pink due to very slow and often incomplete reduction of litmus, with a more intensely coloured upper ring.
M17 broth	Productivity	18 h ± 3 h / 37 °C ± 1 °C	<i>Streptococcus thermophilus</i>	00134	Qualitative	Turbidity (1-2)
NaCl M17 broth	Growth in presence of 6,5 % NaCl	Up to 7 d / 37 °C ± 1 °C	<i>Enterococcus faecalis</i>	00009 00087 00176	Qualitative	Positive reaction: Turbidity
			<i>Streptococcus thermophilus</i>	00134	Qualitative	Negative reaction: No turbidity
MRS broth	Productivity	18 h ± 3 h / 37 °C ± 1 °C anaerobic atmosphere	<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	00102	Qualitative	Turbidity (1-2)
	Growth at 15 °C	Up to 7 d / 15 °C ± 1 °C anaerobic atmosphere	<i>Lactobacillus casei</i>	00100	Qualitative	Positive reaction: Turbidity
			<i>Lacticaseibacillus paracasei</i> (formerly <i>Lactobacillus plantarum</i> )	00104		
			<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	00102	Qualitative	Negative reaction: No turbidity

<sup>a</sup> 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).

<sup>b</sup> 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.

<sup>c</sup> Growth is categorized as 0: no growth; 1: weak growth (partial inhibition); 2: good growth (see ISO 11133).

**Table 1 (continued)**

Medium or reagent	Function	Incubation	Control strains <sup>a</sup>	WDCM numbers <sup>b</sup>	Method of control	Criteria <sup>c</sup>
MRS broth (cont.)	Growth at 45 °C	Up to 7 d / 45 °C ± 1 °C anaerobic atmosphere	<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	00102	Qualitative	Positive reaction: Turbidity
			<i>Lactocaseibacillus paracasei</i> (formerly <i>Lactobacillus plantarum</i> )	00104	Qualitative	Negative reaction: No turbidity
Skimmed milk	Productivity	18 h ± 3 h / 37 °C ± 1 °C anaerobic atmosphere	<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	00102	Qualitative	Growth (1-2)

<sup>a</sup> 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).

<sup>b</sup> 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.

<sup>c</sup> Growth is categorized as 0: no growth; 1: weak growth (partial inhibition); 2: good growth (see ISO 11133).

*Clause 6, first paragraph*

Replace the reference to “ISO 8261 | IDF 122” with “ISO 6887-5”.

*Clause B.4, first paragraph*

Replace the reference to “ISO 8261 | IDF 122” with “ISO 6887-5”.

*Bibliography*

Remove the following reference:

ISO/TS 11133-1, *Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media — Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory*





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