

INTERNATIONAL
STANDARD

ISO
9232

IDF
146

First edition
2003-02-01

AMENDMENT 1
2023-07

**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*



Reference numbers
ISO 9232:2003/Amd.1:2023(E)
IDF 146:2003/Amd.1:2023(E)

© ISO and IDF 2023

STANDARDSISO.COM : Click to view the full PDF of ISO 9232:2003/Amd 1:2023



COPYRIGHT PROTECTED DOCUMENT

© ISO and IDF 2023

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11

Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

International Dairy Federation
Silver Building • Bd Auguste Reyers 70/B
B-1030 Brussels
Phone: +32 2 325 67 40
Fax: +32 2 325 67 41
Email: info@fil-idf.org
Website: www.fil-idf.org

Forewords

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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 document 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).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

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.

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.

IDF (the International Dairy Federation) is a non-profit private sector organization representing the interests of various stakeholders in dairying at the global level. IDF members are organized in National Committees, which are national associations composed of representatives of dairy-related national interest groups including dairy farmers, dairy processing industry, dairy suppliers, academics and governments/food control authorities.

ISO and IDF collaborate closely on all matters of standardization relating to methods of analysis and sampling for milk and milk products. Since 2001, ISO and IDF jointly publish their International Standards using the logos and reference numbers of both organizations.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. IDF shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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).

STANDARDSISO.COM : Click to view the full PDF of ISO 9232:2003/Amd.1:2023

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 ^a	WDCM numbers ^b	Method of control	Criteria ^c		
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				

^a 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).

^b 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.

^c 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 ^a	WDCM numbers ^b	Method of control	Criteria ^c
Glucose MRS broth with overlay agar	Detection of CO ₂ 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>Lactocaseibacillus paracasei</i> (formerly <i>Lactobacillus plantarum</i>)	00104		
			<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	00102	Qualitative	Negative reaction: No turbidity

^a 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).

^b 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.

^c 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 ^a	WDCM numbers ^b	Method of control	Criteria ^c
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)

^a 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).

^b 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.

^c 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*