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AMENDMENT 1
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Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media

AMENDMENT 1

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

AMENDEMENT 1

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Foreword

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For an explanation on 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 the following URL: www.iso.org/iso/foreword.html.

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*.

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

AMENDMENT 1

Introduction

Add the following text as the last paragraph:

When specific standards are revised and new standards developed, they will include a paragraph for performance testing of the culture media used in the standard.

Scope

Replace the last paragraph with the following:

This document also sets criteria and describes methods for the performance testing of culture media. This document is applicable to end-users of ready-to-use media and to producers such as

— commercial bodies producing and/or distributing ready-to-use or semi-finished reconstituted or dehydrated media,

— non-commercial bodies supplying media to third parties, and

— microbiological laboratories preparing culture media for their own use.

3.2.6, electivity of culture medium

Replace the definition with the following:

demonstration, under defined conditions, that non-target organisms, if able to grow on the medium, do not show the same visual characteristics as target microorganisms

4.3.1 General

Add the following text as the third paragraph:

When a formula indicates an ingredient in hydrated form (e.g. $Na_2HPO_4 \cdot 12H_2O$ for BPW in ISO 6887-1) it can be replaced by an anhydrous or hydrated ingredient with a different number of water molecules, as long as the final quantity of the ingredient takes account of this difference by calculation of the molar mass.

4.3.8.1 General

Replace the last sentence with the following:

In all cases, make reference to the appropriate International Standard or the manufacturer's instructions.

5.4.2.5.1.1 Quantitative testing

Replace the first two paragraphs with the following:

For the quantitative enumeration test, a level of approximately 100 cfu is necessary to achieve sufficient precision (see Table 1). This can necessitate the use of more than one plate.

A practicable range of 80 cfu to 120 cfu per plate with a minimum number of 50 cfu per plate should be used. The use of more than one plate will increase the precision. For filters, the same number of cfu is needed using one or more filters. Table 1 shows the 95 % confidence intervals associated with colony counts.

5.4.2.5.1.2 Qualitative testing

Replace the part of the sentence introducing the list with the following:

The volume of suspension used for testing should contain

5.4.2.5.2 Inoculum level for selectivity testing

Replace the sentence with the following:

For selectivity testing of culture media, a suspension of the non-target microorganism containing at least 10^4 cfu is inoculated on to the plate or into the tube of medium.

5.4.2.5.3 Inoculum level for specificity testing

Replace the sentence with the following:

For qualitative tests of plate media, for specificity an inoculum level of at least 10^3 cfu is needed.

7.3 Testing of culture media used for membrane filtration

Add the following text as the last paragraph:

When testing with membrane filters, if the criteria in Table F1 are not achieved, the laboratory should assess the discrepancies between the results.

8.3.2 Procedure

Replace the fourth list item with the following:

— **Inoculation of non-target microorganisms:** Inoculate one tube of test broth per microorganism with an inoculum containing a higher number (at least 10^4 cfu) and mix.

Replace the seventh, eighth and ninth list items with the following:

— Remove one loopful (10 µl) from the tube containing the target organism and streak on a plate of the relevant selective medium (e.g. XLD).

— Remove one loop (10 µl) from the culture of non-target microorganism and streak on a plate of a non-selective medium (e.g. TSA).

— If a mixed culture of target and non-target organisms has been used, remove one loop (10 µl) and streak on a plate of the specific medium for the target microorganism (e.g. XLD).

Replace the last paragraph with the following:

If a larger volume of medium is used (e.g. 225 ml), the user may choose to adjust the volume of inoculum proportionately, but without changing the total number of organisms inoculated.

8.3.3 Calculation and interpretation of results

Replace the last paragraph with the following:

Selectivity of the liquid test broth is satisfactory if either no growth, or less than 10 cfu or less than 100 cfu of non-target microorganisms occurs on the non-selective agar plate, as specified in Annexes E and F or the specific International Standard.

8.4.2.2 Confirmation media

Replace the first list item with the following:

— For performance testing of liquid confirmation media, inoculate the medium under test with ≥ 10^4 cfu of the target organism (for example, by using a working culture suspension containing more than 10^6 cfu/ml and a 10 µl loop).

After 8.4.3

Add the following text as a new subclause:

8.5 Multipurpose liquid media

For multipurpose liquid media such as BPW, perform a qualitative pre-enrichment test as a minimum, using a pathogenic organism appropriate to the laboratory's range of test methods, e.g. *Salmonella.*

Commercial and non-commercial suppliers of multipurpose liquid media are also expected to test these multipurpose liquid media as diluents for enumerations of microorganisms to further ensure the quality of the culture media they supply.

If a new or revised standard uses a multipurpose medium as part of the isolation procedure for a target organism not currently included in this document (ISO 11133:2014) or specific standards published subsequently, the performance testing described in the new or revised standard for the multipurpose medium should at least include the use of the target organism.

C.4 Qualitative single tube method for selective liquid enrichment media (with target, non-target, or a mixture of target and non-target microorganisms in the same tube) (see 8.3 and Figure C.3)

In the middle and right-hand columns, replace "≥ 10^3" with "≥ 10^4".

In the middle and right-hand columns, delete the text in brackets in the bottom boxes.

Annex E (page 45)

Replace footnote m with the following:

If BPW is used for more than one application, perform at least one enrichment test as a minimum using a pathogenic organism appropriate to the laboratory's range of test methods, e.g. *Salmonella.* See 8.5.

Table E.1, Selective media for enumeration of microorganisms

On page 47, delete the row "IS ("TS")".

Modify the row "TBX – Productivity" as follows:

TBX	S	β-D-Glucuronidase positive *Escherichia coli*	ISO 16649-1 and ISO 16649-2	Productivity	(21 ± 3) h / (44 ± 1) °C	*Escherichia coli*[d,h]	00012 00013	TSA	Quantitative	$P_R \geq 0,5$	Blue colonies
						Escherichia coli[h]	00202[b]				

For the row "TSC (SC)", "Productivity", column "Reference media", replace "TSA or other nonselective medium for anaerobes" with "A suitable nonselective medium for anaerobes ($P_R \geq 0,5$) or media batch TSC (SC) already validated ($P_R \geq 0,7$)".

Table E.1, Non-selective media for enumeration of microorganisms

Add, on page 50, the row "IS ("TS")" that was deleted from *Table E.1, Selective media for enumeration of microorganisms*, with modifications for columns "Reference media" and "Criteria" as follows:

IS ("TS")	S	Sulfite-reducing bacteria	ISO 15213	Productivity	(24 ± 3) h to (48 ± 2) h/ (37 ± 1) °C anaerobic atmosphere	*Clostridium perfringens*	00007[b] 00080	A suitable non-selective medium for anaerobes	Quantitative	$P_R \geq 0,7$	Black colonies
				Specificity		*Escherichia coli*[d]	00012 00013	—	Qualitative	—	No blackening

Table E.1, Selective enrichment media

For the row "ITC", "Selectivity", column "WDCM numbers", add a footnote to "00023[b]".

Table E.1, Selective isolation media

For the row "CIN/SSDC", column "Incubation" replace "(21 ± 3) h/(30 ± 1) °C" with " (24 ± 2) h/(30 ± 1) °C".

Modify the row "TBXj – Productivity" as follows:

TBXj	S	β-D-Glucuronidase positive *Escherichia coli*	ISO 16649-3	Productivity	(21 ± 3) h/ (44 ± 1) °C	*Escherichia coli*[d,h]	00012 00013	—	Qualitative	Good growth (2)	Blue colonies
						Escherichia coli[h]	00202[b]				

Table E.1, footnotes

Replace footnote m with the following:

If BPW is used for more than one application, perform at least one enrichment test as a minimum using a pathogenic organism appropriate to the laboratory's range of test methods, e.g. *Salmonella.* See 8.5.

Annex F (page 67)

Replace footnote k with the following:

If BPW is used for more than one application, perform at least one enrichment test as a minimum using a pathogenic organism appropriate to the laboratory's range of test methods, e.g. *Salmonella.* See 8.5.

Table F.1, Selective media for enumeration of microorganisms by comparing with a non-selective reference medium

For the row "Colilert-18", column "Control strains", replace "*Klebsiella pneumoniae*" with "*Klebsiella variicola*" [WDCM 00206].

For the row "GVPC", "Selectivity", column "Control strains", delete "*Pseudomonas aeruginosa*[d]", and column "WDCM numbers", delete "00026 or 00025".

Delete the row for "Sulfite Iron/Tryptose Sulfite (TS)".

For the row "TSC", column "Reference medium", replace "TSA or Blood agar or other nonselective medium for anaerobes" with "A suitable non-selective medium for anaerobes".

Table F.1, Selective media for enumeration of microorganisms by comparing with a previously accepted batch (for use in special cases)

For the row "Colilert-18", column "Control strains", replace "*Klebsiella pneumoniae*" with "*Klebsiella variicola*" [WDCM 00206].

Delete the row for "Sulfite Iron/Tryptose Sulfite (TS)".

Table F.1, Non-selective media for enumeration of microorganisms

Add, on page 72, a row for "Sulfite Iron/Tryptose Sulfite (TS)" corresponding to the two rows that were deleted from Table F.1, *Selective media for enumeration of microorganisms by comparing with a non-selective reference medium* and from Table F.1, *Selective media for enumeration of microorganisms with a previously accepted batch* with modifications for columns "Incubation", "Reference media" and "Criteria" as follows:

Sulfite Iron Tryptose Sulfite (TS)	S	Sulfite-reducing anaerobes (clostridia)	ISO 6461-2	Productivity	(20 ± 4) h to (44 ± 4) h / (37 ± 1) °C anaerobic atmosphere	*Clostridium perfringens*	00007[b] 00080	A suitable non-selective medium for anaerobes or Media batch Sulfite iron already accepted	Quantitative	$P_R \geq 0,7$	Black colonies
				Specificity		*Escherichia coli*[d]	00012 00013	—	Qualitative	—	No blackening