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SIST EN 17710:2025

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Rastlinski biostimulanti - Ugotavljanje prisotnosti Listeria monocytogenes

Plant biostimulants - Detection of Listeria monocytogenes

Pflanzen-Biostimulanzien - Nachweis von Listeria monocytogenes

Biostimulants des végétaux - Recherche de Listeria monocytogenes

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65.080	Gnojila	Fertilizers

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Plant biostimulants - Detection of *Listeria monocytogenes*

Biostimulants des végétaux - Recherche de *Listeria monocytogenes*

Pflanzen-Biostimulanzien - Nachweis von *Listeria monocytogenes*

This European Standard was approved by CEN on 26 August 2024.

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Contents

Page

European foreword 3
Introduction 4
1 Scope 5
2 Normative references 5
3 Terms and definitions 5
4 Principle 6
5 Culture media, reagents, antisera 7
6 Equipment and consumables 7
7 Sampling 7
8 Preparation of test sample 8
9 Expression of results 13
10 Performance characteristics of the method 13
11 Test report 14
12 Quality assurance 14
Annex A (normative) Diagram of the procedures 15
Annex B (normative) Composition and preparation of culture media and reagents 16
Annex C (informative) Method validation study and performance characteristics 26
Annex ZA (informative) Relationship of this European Standard and the essential requirements of Regulation (EU) 2019/1009 laying down rules on the making available on the market of EU fertilising products aimed to be covered 29
Bibliography 30

European foreword

This document (EN 17710:2024) has been prepared by Technical Committee CEN/TC 455 “Plant Biostimulants”, the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by May 2025, and conflicting national standards shall be withdrawn at the latest by May 2025.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document supersedes CEN/TS 17710:2022.

EN 17710:2024 includes the following significant technical changes with respect to CEN/TS 17710:2022:

— The European foreword and the introduction have been updated;

— The Normative references reported in Clause 2 have been updated;

— The definitions in Clause 3 have been revised;

— Clauses 8 and 9 have been combined and the text has been revised;

— Annexes A, B, and C have been revised;

— Annex ZA has been added.

This document has been prepared under a standardization request addressed to CEN by the European Commission. The Standing Committee of the EFTA States subsequently approves these requests for its Member States.

For the relationship with EU Legislation, see informative Annex ZA, which is an integral part of this document.

Any feedback and questions on this document should be directed to the users’ national standards body. A complete listing of these bodies can be found on the CEN website.

According to the CEN-CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Türkiye and the United Kingdom.

Introduction

The European Committee for Standardization (CEN) was requested by the European Commission (EC) to draft European Standards or European Standardization deliverables to support the implementation of Regulation (EU) 2019/1009 of 5 June 2019 [1] laying down rules on the making available on the market of EU fertilising products ("FPR" or "Fertilising Products Regulation").

This standardization request, presented as SR M/564 and relevant amendments, also contributes to the Communication on "Innovating for Sustainable Growth: A Bio economy for Europe".

The interest in plant biostimulants has increased significantly in Europe as a valuable tool to use in agriculture. Standardization was identified as having an important role in order to promote the use of biostimulants. The work of CEN/TC 455 seeks to improve the reliability of the supply chain, thereby improving the confidence of farmers, industry, and consumers in biostimulants, and will promote and support commercialisation of the European biostimulant industry.

WARNING — Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this document be carried out by suitably trained staff.

1 Scope

This document specifies a method for the detection of *Listeria monocytogenes* in microbial plant biostimulants.

This document is applicable to the blends of fertilizing products where a blend is a mix of at least two of the following component EU fertilising products categories: Fertilizers, Liming Materials, Soil Improvers, Growing Media, Plant Biostimulants and where the following category Plant Biostimulants is the highest percentage in the blend by mass or volume, or in the case of liquid form by dry mass. If Plant Biostimulants is not the highest percentage in the blend, the European Standard for the highest percentage of the blend applies. In case a blend of fertilizing products is composed of components in equal quantity or in case the component EU fertilising products used for the blend have identical formulations[1], the user decides which standard to apply.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 17724:2024, *Plant biostimulants — Terminology*

EN 17708:2024, *Plant biostimulants — Preparation of sample for microbial analysis*

EN 17702-1:2024, *Plant biostimulants — Sampling and sample preparation — Part 1: Sampling*

EN ISO 7218:2024, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

EN ISO 11133:2014,[2] *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*

EN ISO 11290-1:2017, *Microbiology of the food chain — Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. — Part 1: Detection method (ISO 11290-1:2017)*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 17724:2024 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at https://www.electropedia.org/

— ISO Online browsing platform: available at https://www.iso.org/obp

[1] An example of such a blend is a product with 2 claimed functions consisting of a non-microbial plant biostimulant and an organic fertilizer composed of 1 kg/kg of plant biostimulant from seaweed.
[2] As impacted by EN ISO 11133:2014/A1:2018 and EN ISO 11133:2014/A2:2020.

3.1
Listeria monocytogenes
microorganisms which form typical colonies on solid selective media and which display the morphological, physiological and biochemical characteristics described when tests are carried out in accordance with this document

Note 1 to entry: *L. monocytogenes* is a Gram-positive, non-spore forming, rod-shaped bacterium that belongs to the genus *Listeria*, phylum *Firmicutes*.

Note 2 to entry: *L. monocytogenes* is a ubiquitous bacterial pathogen that causes serious localized and generalized infections in humans.

Note 3 to entry: *L. monocytogenes* are catalase positive and oxidase negative. They produce flagella when grown at a temperature between 20 °C and 25 °C but not at 37 °C. They produce a β-hemolysin on blood agar plates, which is part of the CAMP (Christie, Atkins, and Munch-Petersen) diagnostic test.

Note 4 to entry: *L. monocytogenes* can grow at temperatures between −0,4 °C and 50 °C, with an optimum temperature of 30–37 °C. They can withstand freezing, but they are inactivated by heating at 60 °C for 30 min.

Note 5 to entry: *L. monocytogenes* are facultative anaerobes that utilize glucose, lactose, and rhamnose under aerobic conditions and can ferment several hexoses and pentoses under anaerobic conditions.

Note 6 to entry: *L. monocytogenes* can grow over a pH range of 4–9.5 and a water activity of 0.90 to 0.97. They can also grow in 10 % sodium chloride.

[SOURCE: EN ISO 11290-1:2017, 3.1]

4 Principle

4.1 General

The detection of *Listeria monocytogenes* requires four successive stages as specified in Annex A.

NOTE *L. monocytogenes* can be present in small numbers and is often accompanied by a considerably larger number of bacteria belonging to different taxonomic groups or different *Listeria* species. Pre-enrichment is used to permit the detection of low numbers of *L. monocytogenes* or injured *L. monocytogenes* [2].

4.2 Pre-enrichment in selective liquid medium

Half-Fraser broth (225 ml) at ambient temperature shall be inoculated with the test portion (25 g or 25 ml), then shall be incubated at 30 °C ± 2 °C for 24 h to 26 h.

For large quantities (e.g. 1 l or more), it is recommended to pre-warm the broth to 30 °C before mixing it with the test portion.

4.3 Enrichment in/on selective media

Fraser broth shall be inoculated at 37 °C (0,1 ml of culture in 10 ml of Fraser broth) and shall be incubated at 37 °C ± 2 °C for 24 h ± 2 h.

4.4 Plating out on selective solid media

Both primary and secondary enrichments shall be streaked onto:

— Agar Listeria according to Ottaviani and Agosti (ALOA) [3];

— A second selective agar of choice, e.g. PALCAM agar, Oxford agar.

The agar prepared according to Ottaviani and Agosti shall be incubated for 48 h ± 2 h at 37 °C ± 2 °C and then shall be examined. The second selective agar shall be incubated as specified by the manufacturer.

4.5 Confirmation

Colonies of presumptive *L. monocytogenes* shall be subcultured and their identity shall be confirmed by means of appropriate morphological and biochemical tests.

5 Culture media, reagents, antisera

Current laboratory practices shall refer to EN 17708:2024 and EN ISO 11133:2014[2].

Composition of culture media and reagents and their preparation are described in Annex B.

6 Equipment and consumables

Disposable equipment is an acceptable alternative to reusable glassware if it has suitable specifications.

Usual microbiological laboratory equipment specified in EN ISO 7218:2024 shall be used and, in particular, the following.

6.1 Apparatus for dry sterilization (oven) or wet sterilization (autoclave).

6.2 Drying cabinet or incubator, capable of operating between 25 °C and 50 °C.

6.3 Incubators, capable of operating at 30 °C ± 2 °C, 37 °C ± 2 °C, and at 25 °C ± 2 °C (optional).

6.4 Water bath, capable of operating at 47 °C ± 2 °C.

6.5 Sterile loops, approximately 3 mm in diameter or 10 µl, and inoculating needle or wire.

6.6 pH-meter, having a maximum permissible error of ± 0,1 pH unit at 25 °C.

6.7 Sterile graduated pipettes or automatic pipettes of nominal capacities of 1 ml, and 10 ml.

6.8 Sterile Petri dishes, with a diameter of approximately 90 mm and (optional) large size (diameter approximately 140 mm).

6.9 Microscope, preferably with phase-contrast, and with slides and cover slips.

6.10 Refrigerator, capable of operating at 5 °C ± 3 °C.

6.11 Peristaltic blender (Stomacher®[3]) with 400 ml sterile bags.

6.12 Blender motor and jars or vortex.

7 Sampling

Sampling is not part of the method specified in this document. A representative sample of the product shall be obtained as specified in EN 17702-1:2024.

It is important that the laboratory receives a sample which is representative and has not been damaged or changed during transport or storage.

[3] Stomacher® is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.

8 Preparation of test sample

8.1 General

Refer to EN 17708:2024 for general rules for the preparation of the initial suspension for microbiological examination. To prepare the analytical unit, liquids or free flowing materials shall be agitated until the contents are homogeneous. If the sample is solid, the analytical unit shall be obtained by taking a portion from several locations within the sample. To reduce the workload, the analytical units may be combined for analysis. It is recommended that a composite contains no more than five analytical units.

The initial suspension of the microbial biostimulant product samples shall be prepared according to EN ISO 11290-1:2017 using, as dilution fluid, the selective primary enrichment medium, half-Fraser broth, described in B.2.

A representative sample of the product shall be prepared considering the different formulations of plant biostimulants.

A portion of 25 g or 25 ml of the product shall be added to 225 ml of half-Fraser broth in a 400 ml sterile bag (6.11) or a blender jar (6.12). For composite samples, analytical units may be combined up to 125 g or ml (e.g. 125 g or 125 ml of biostimulant to 1 125 ml of half-Fraser broth). The half-Fraser broth shall be pre-warmed to room temperature before use.

If alternate analytical units are required, a ratio of 1 part sample material to 9 parts half-Fraser broth shall be maintained.

To minimize the effect of the sampling on the significance and the reliability of the analytical results, the number of replicates tested shall be increased to 5.

8.2 Liquid (water-based) formulations

A portion of 25 ml of the product (the analytical unit) shall be aseptically added to 225 ml of half-Fraser broth in a 400 ml sterile bag (6.11) or a blender jar (6.12). The half-Fraser broth shall be pre-warmed to room temperature before use. The sample shall be blended, stomached or vortexed as required for thorough mixing.

8.3 Liquid (oil-based) emulsifiable concentrate (EC) formulations

8.3.1 General

A portion of 25 ml of the product (the analytical unit) shall be aseptically added to 225 ml of half-Fraser broth in a 400 ml sterile bag (6.11) or a blender jar (6.12). The half-Fraser broth shall be pre-warmed to room temperature before use. The sample shall be blended, stomached or vortexed as required for thorough mixing.

8.3.2 Solid wettable powder (WP) formulations

A portion of 25 g of the product (the analytical unit) shall be aseptically added to 225 ml of half-Fraser broth in a 400 ml sterile bag (6.11). The half-Fraser broth shall be pre-warmed to room temperature before use. The mixture shall be homogenized for 2 min at highest speed with a peristaltic blender (6.11).

8.3.3 Solid water dispersible granules (WDG) formulations

A portion of 25 g of the product (the analytical unit) shall be aseptically added to 225 ml of half-Fraser broth in a 400 ml sterile bag (6.11). The half-Fraser broth shall be pre-warmed to room temperature before use. The mixture shall be homogenized for 2 min at highest speed with a peristaltic blender (6.11).

8.3.4 Solid pellets, granules, microgranules (slow release) formulations

A portion of 25 g of the product (the analytical unit) shall be aseptically added to 225 ml of half-Fraser broth in a 400 ml sterile bag (6.11). The half-Fraser broth shall be pre-warmed to room temperature before use. The mixture shall be homogenized for 2 min at highest speed with a peristaltic blender (6.11).

8.3.5 Solid substrates

A portion of 25 g of the product (the analytical unit) shall be aseptically added to 225 ml of half-Fraser broth in a 400 ml sterile bag (6.11). The half-Fraser broth shall be pre-warmed to room temperature before use. The mixture shall be homogenized for 2 min at highest speed with a peristaltic blender (6.11).

8.4 Non-selective pre-enrichment

The test portion sample (25 g or 25 ml) shall be prepared in half-Fraser broth (225 ml). The incubation shall be done for 25 h ± 1 h at 30 °C ± 2 °C.

NOTE 1 A black coloration can develop during incubation.

NOTE 2 After the incubation, it is possible to store the pre-enriched sample at 5 °C (6.10) before transfer to Fraser broth for a maximum of 72 h.

8.5 Selective enrichment

8.5.1 After incubation of the initial suspension (primary enrichment in half-Fraser broth) for 25 h ± 1 h, 0,1 ml of the culture obtained in 8.4 shall be transferred to a tube or bottle containing 10 ml of secondary enrichment medium (Fraser broth) (described in B.3).

8.5.2 The inoculated medium (8.5.1) shall be incubated for 25 h ± 1 h at 37 °C ± 2 °C (6.3).

Half-Fraser broth and Fraser broth may be refrigerated before transfer or isolation on selective agar for a maximum of 72 h. Refrigeration provides greater laboratory productivity and analytical flexibility. Following the period of refrigeration, the secondary enrichment broth shall always be resuspended before transfer or plating onto agar media.

8.6 Plating out

8.6.1 General

8.6.1.1 From the primary enrichment culture (8.4) incubated for 25 h ± 1 h at 30 °C ± 2 °C (6.3), shall inoculate the surface of the first selective plating medium, Agar Listeria according to Ottaviani and Agosti (ALOA) (described in B.4), by means of a loop (6.5), to obtain well-separated colonies.

Proceed in the same way with the second selective plating-out medium of choice (B.5).

NOTE Half-Fraser broth and Fraser broth can be refrigerated at 5 °C (6.10) before isolation on selective agar for a maximum of 72 h [4].